PRODUCTION ECOLOGY OF GREEN MACROALGAL MATS (*ENTEROMORPHA* SPP.) IN THE COOS BAY, OREGON ESTUARY

by

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A DISSERTATION

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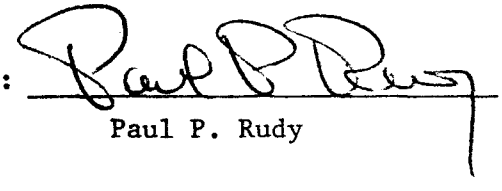
APPROVED: 

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Title: PRODUCTION ECOLOGY OF GREEN MACROALGAL MATS (ENTEROMORPHA SPP.) IN THE COOS BAY, OREGON ESTUARY

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Many tideflats in the Coos Bay, Oregon estuary support seasonally abundant populations of green macroalgae, primarily Enteromorpha and Ulva spp. (Chlorophycophyta, Ulvales). This study examined the production ecology of these algae by a combination of field and laboratory measurements in order to determine algal photosynthetic responses under typically occurring estuarine conditions. While benthic algae are often considered to be of secondary importance in estuarine production budgets the results of the present study show that algal mats can be at least as productive as salt marshes and eelgrass beds.

Monthly field collections of Enteromorpha populations indicated a maximum standing crop in August in both 1981 and 1982. From April through September, algal abundance progressed up the mudflat from low-to mid-intertidal elevations until storms in fall and winter physically

stripped algae from the tideflats.

Potential algal mat production was estimated with a computer simulation which integrated (1) field measurements of standing crop, salinity, light, and temperature (2) laboratory measurements of photosynthetic rates under estuarine conditions, and (3) computer-generated estimates of tidal exposure and submergence. For the growing season of 1982, algal production is estimated to be 2650 g dry wt m^{-2} over the entire field study site. Submerged photosynthesis accounted for an average of 95% of the total production.

The release of photosynthate as dissolved organic carbon (DOC) was quantified during laboratory determinations of photosynthetic rates. About 5% of ^{14}C-carbon fixed during normal submerged photosynthesis is lost as DOC. Sephadex G-15 gel fractionation of this DOC suggests that most of the released material consists of oligosaccharides and amino acids. These materials derive from intracellular pools with high turnover rates. Estuarine fluctuations of tidal exposure, rainfall, and reduced salinity increase the amount of DOC released during photosynthesis; gel fractionation of this DOC produces an elution pattern which closely resembles that of the ethanol-soluble cellular metabolites, plus some larger substances. Heterotrophic microbes in the estuary quickly utilize this photosynthetically derived material. Thus, much of the total algal production enters the estuarine trophic structure before the formation of detritus from senescent algae.

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To Nancy

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

Estuaries have historically been very important in the development of human commerce and culture. One reason for this, and an area of continuing public interest, is the harvest of fish and shellfish species that inhabit estuaries for some part of or all of their lives. Such animals are able to grow abundantly due to the high primary productivity of the plants that live in these places where rivers meet the ocean.

Plants take inorganic forms of carbon, oxygen, and nitrogen and photosynthetically produce complex organic materials, which are in turn consumed by rank on rank of heterotrophic creatures, including people. Estuarine waters have higher concentrations of inorganic nutrients than do those of open oceans. This is due to an influx of nutrients from coastal waters that may experience upwelling, to the trapping of nutrients that are transported from the drainage basin by the estuary's river system, and to the input of nutrients in domestic sewage. These nutrients, and other materials, are distributed throughout the estuary by a combination of river flow and tidal action.

Microbial decomposition of organic matter can rapidly regenerate inorganic nutrients within estuaries. Abundant populations of aerobic and anaerobic microbes inhabit the water column and the sediments of widespread, organic-rich mudflats. Estuarine macrophytes enhance the deposition of sediments and particulate organic materials by physically

baffling the moving water. The reduced current speeds in salt marshes, seagrass beds, and benthic algal mats permit suspended particulates to settle out (e.g., Frostick and McCave, 1978; Fonseca et al., 1982).

Primary production in estuaries has been divided into six components (Correll, 1978). The three guilds of vascular plant producers include beds of seagrasses, salt marshes, and plants in the upland system whose production is brought into the estuary by runoff. The three algal components are the phytoplankton, the microscopic periphyton (which may functionally include the photosynthetic bacteria), and the benthic macroalgae. Of these six groups, the salt marshes, the seagrass beds, and the phytoplankton are usually considered to be of greatest importance, a conclusion that is based on the great area that may be occupied by these plants within temperate, Atlantic estuaries and on their potentially very high biomass.

However, it has recently been found that marine macroalgae also can be extremely productive (e.g., Mann, 1972; Littler and Murray, 1974). Consideration of standing crop values alone may cause unwitting underestimation of potential production. It is through high biomass turnover rates that such algae contribute large amounts of photosynthetically produced organic matter to estuarine and coastal habitats (Hatcher et al., 1977). Indeed, much of the algal material is consumed by grazers and particle feeders long before the algal population appears to decline; this contrasts with the salt marsh paradigm, in which it is assumed that production becomes available after the formation of particulate detritus and export to the rest of the estuary (Odum and de la Cruz, 1967).

The Coos Bay, Oregon estuary has about 10% of its historically known salt marsh remaining (Hoffnagle and Olson, 1974). Most of what presently exists occurs only in the very high intertidal zone, where exchange of materials with the rest of the estuary is diminished by infrequent tidal submergence. Additionally, salt marshes may be "self-ish", retaining much of their production within the marsh soil (Haines, 1977). There are many eelgrass beds in Coos Bay, primarily of Zostera marina, although the small introduced species Z. japonica is increasing in its intertidal distribution. However, from 10 to 70% of the standing crop in these eelgrass beds consists of associated green algae (Gonor et al., 1979). These same green algae, primarily of Enteromorpha and Ulva spp. (Chlorophycophyta, Ulvales), form seasonally abundant mats on numerous intertidal flats.

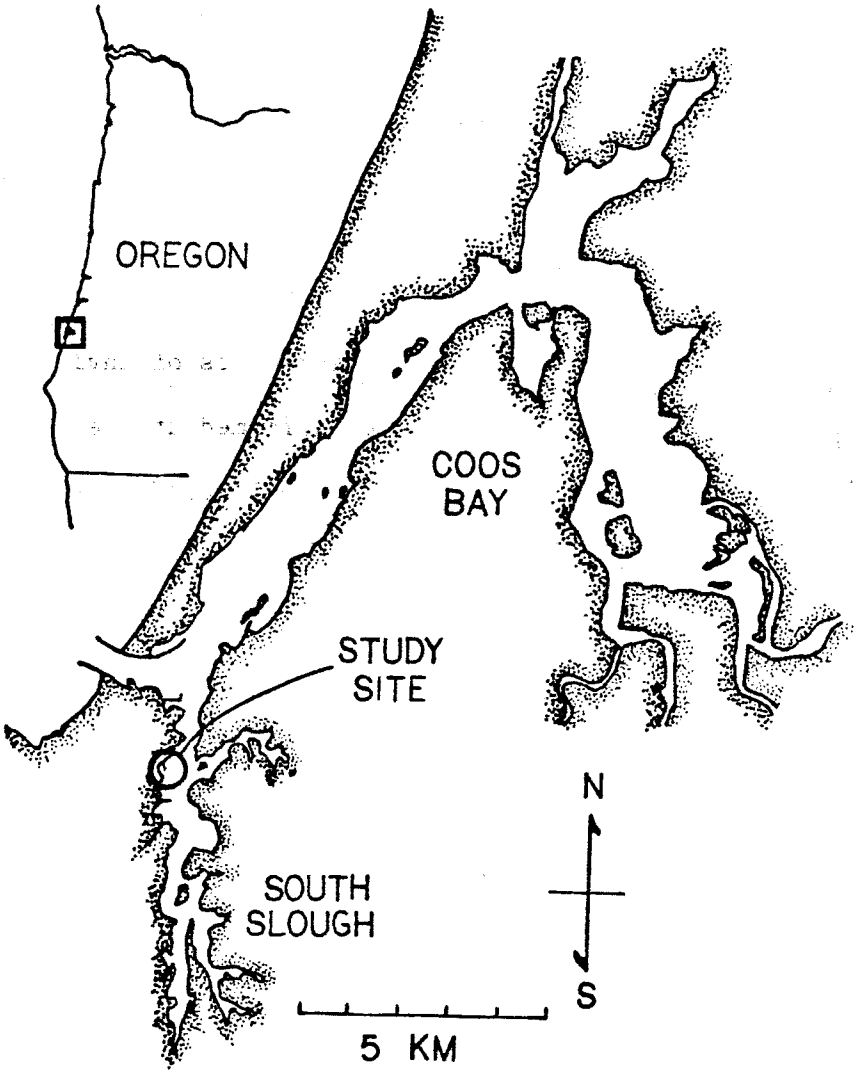
Such green algae are known for their opportunistic growth in disturbed or variable marine and estuarine habitats. They are capable of sustaining high photoysnthetic rates while submerged (King and Schramm, 1976; Arnold and Murray, 1980; Littler and Littler, 1980). The isomorphic generations reproduce quite frequently (Christie and Evans, 1962) by liberating motile gametes and zoospores (Lersten and Voth, 1960; Christie and Shaw, 1968; Jones and Babb, 1968; Woodhead and Moss, 1975). Enteromorpha spp. in particular are quite resistant to domestic and industrial pollution (Edwards, 1972; Tewari, 1972; Coleman and Stewart, 1979), and they tolerate a wide range of salinities (Black, 1971; Reed and Russell, 1978, 1979). In short, these algae should have a competitive advantage over other algae under varying estuarine conditions.

The green algae most commonly encountered on tideflats in Coos Bay are the filamentous, branching species Enteromorpha prolifera and E. clathrata, and the sheet-like E. linza and Ulva spp. E. prolifera appeared as an early and persistant dominant in the algal blooms of 1981 and 1982; therefore, most of the experimental measurements of photosynthesis were performed with this species. The filamentous algae have a hollow central axis that is filled with water and gases. Rising tides lift the buoyant axis off the mudflat, and the many side branches are displayed horizontally in the tidal current close to the water's surface. Sheet-like species are not buoyant and remain as an understory to the filamentous algae.

The fundamental purpose of the present study was to determine the production of these green algal mats in the Coos Bay, Oregon estuary. I approached this topic with a combination of (1) observations on the species composition, spatial distribution, and abundance of one algal mat in the South Slough arm of the Coos Bay estuary (Figure 1), (2) field measurements of environmental factors that might influence algal production, and (3) laboratory measurements of the photosynthetic responses of the algae under a range of conditions observed in the estuary. The field study site was chosen for its proximity to the Oregon Institute of Marine Biology and because observations of the area for a number of years before this study indicated that South Slough tideflats often support populations of benthic green macroalgae.

It has generally been assumed that estuarine macrophyte production must break down into detrital particles before becoming available as food for estuarine animals (Odum and de la Cruz, 1967). However,

Figure 1. Location of the field study site in the South Slough branch of the Coos Bay, Oregon estuary. Measurements of environmental factors and algal standing crop changes were performed at the site, and algal material for laboratory experiments was collected at the site.


OREGON
COOS
BAY
STUDY
SITE
SOUTH
SLOUGH
N
S
5 KM

there is increasing evidence that marine algae lose some of their photosynthetically fixed materials as dissolved organic carbon (DOC) before detrital formation (e.g., Khailov and Burlakova, 1969; Sieburth, 1969; Moebus and Johnson, 1974; Brylinsky, 1977). Therefore, one aim of the present research was to determine if any of the green algal production enters the estuary as DOC. If measurable DOC release occurred, additional aims were to determine how fluctuating estuarine conditions affected such release and to identify the major substances in the released material.

It has been noted that measurement of macroalgal standing crop may underestimate actual production (Mann, 1972; Brinkhuis, 1977; Hatcher et al., 1977). Therefore, another aim of this research was to contrast production as indicated by maximum standing crop with production as estimated by simulation of growth functions with limits set by in situ conditions. Additionally, I wanted to estimate how the total algal production entered the estuarine system in various forms besides DOC release.

CHAPTER TWO

RELEASE OF DISSOLVED ORGANIC CARBON

Marine macrophytes photosynthetically fix large quantities of carbon into organic substances. While it is assumed that most of this material becomes available to the aquatic community as particulate detritus some time after synthesis, much of it enters the environment as dissolved organic carbon (DOC) both during photosynthesis and following senescence (Khailov and Burlakova, 1969; Sieburth, 1969; Moebus and Johnson, 1974; Brylinsky, 1977).

The estuarine production components of phytoplankton, salt marshes, and seagrasses have been found to release DOC (Gallagher et al., 1976; Fogg, 1977; Penhale and Smith, 1977), as have many macroalgae (Khailov and Burlakova, 1969; Sieburth, 1969; Moebus and Johnson, 1974). The intertidal habitat of Enteromorpha spp. mats in Coos Bay subjects them to the typical fluctuations of repeated exposure and resubmergence, potential desiccation and rainfall stress, and variable estuarine salinity. These factors affect photosynthetic rates and DOC release in some marine macrophytes (Sieburth, 1969; Johnson et al., 1974; Moebus et al., 1974; Penhale and Smith, 1977; Quadir et al., 1979; Gordon et al., 1980). The purposes of this portion of the present study were to quantify the release of DOC from actively photosynthesizing thalli of E. prolifera, with particular attention to the above-mentioned environmental fluctuations, and to investigate the potential for use of this DOC by heterotrophic microbes.

Materials and Methods

Algal Material

Samples of Enteromorpha prolifera, one of the more abundant species of Enteromorpha in Coos Bay, were collected from mudflats in the field study site in South Slough (Figure 1), at +2.0 feet (0.6 meters) above mean lower low water (MLLW) during the period of August to October 1981. Thalli were gently rinsed in sea water to remove sediments, small grazers, and epiphytes, and then held overnight in aerated, ambient-temperature sea-water aquaria.

Photosynthesis and DOC Release

For determinations of photosynthetic rates and DOC release rates, algae were incubated in either 300-ml bottles or 1.5-liter Plexiglas chambers with magnetic stir bars. Each Plexiglas chamber had two stoppers through which fluids could be injected or withdrawn by syringe and an insert grid to which the algae were attached.

Submerged Photosynthesis and DOC Release

Gently blotted algae of known fresh weight were placed in the incubation vessels, and sterilized synthetic sea water (Rila Sea Salts) of the required salinity was then added. The dissolved carbon dioxide, bicarbonate, and carbonate concentrations of the water were determined beforehand using the techniques of Strickland and Parsons (1968). The average algal density over all experiments was 0.29 g dry wt liter^{-1}.

Bottles and chambers were maintained at 16° C, the predominant summer water temperature in the bay, in a water bath. 20 uCi of $NaH^{14}CO_3$ (New England Nuclear) in 1 ml were added to the 1.5-liter chambers, and 4 uCi to the 300-ml bottles, with a final specific activity of about 0.82 uCi mg^{-1} dissolved inorganic carbon. All incubations were performed outside between 11 a.m. and 3 p.m. on clear, sunny days in early fall to ensure that light intensities would be above saturation (King and Schramm, 1976; Arnold and Murray, 1980). Dark controls were wrapped in foil, and samples were taken just after the introduction of label to establish initial background activities.

At the end of the 3-hour incubation, algae were fixed for 1 hour in 100 ml of 5% formalin in 30 $^{o}/oo$ sea water adjusted to pH 2.0 with HCl. Following dry weight determination, the algae were ground to a fine powder, and the activities of aliquots of 10 to 30 mg were counted by liquid scintillation. Aliquots of the algal fixative were also counted, for some activity leached from the algae. Each sample was counted three times for either 15 minutes or to 1.5% accuracy on a Beckman LS 150 Scintillation Counter. Corrections for quench were made using standard curves of percent counting efficiency versus external standards ratio.

At specified intervals during the incubations, 3-ml water samples were removed from the vessels and acidified to pH 2 in scintillation vials with 0.5 ml of the algal fixative previously described. These were then flushed for 10 minutes with CO_2-free air. Controls indicated that less than 30 ^{14}C-decays per minute (DPM) above background of inorganic label remained after flushing. The resulting acid-stable

^{14}C activity is the experimental measure of DOC.

Photosynthesis in Air

For measurements of photosynthesis in air, a modification of the procedure of Darley et al. (1976) was used. Desiccated algal sections were placed in the 1.5-liter chambers with 20 uCi of tracer in 1 ml in a cup, after which the chambers were equilibrated in the water bath, sealed, and $^{14}CO_2$ liberated by the addition of 1 ml of 85% lactic acid to the cup. After 20 minutes, the algal pieces were removed and subdivided. One half of each section was fixed, and carbon uptake was determined as before. The other half was immersed in 100 ml of synthetic sea water for one hour, and DOC release was measured.

Results

In seven three-hour incubations of Enteromorpha prolifera samples in 30 $^{o}/oo$ sea water, net carbon fixation averaged 7.37 mg C g dry wt^{-1} h^{-1} in the light, and DOC release averaged 0.26 mg C g dry wt^{-1} h^{-1}, giving a mean of 3.5% of recently fixed carbon lost (Table 1). Dark fixation and release rates were both less than 1% of the light rates. Figure 2 shows the time course of the accumulation of DOC for one of the incubations. Over the three hours of the experiment, DOC accumulation appears to be linear, with a constant release rate.

Portions of the algal mat that are left exposed by receding tides may become desiccated on dry, sunny, or windy days. As these algae become increasingly desiccated, their photosynthesis drops quickly (Figure 3), until they are barely fixing carbon after 50%

Table 1. Enteromorpha prolifera: carbon fixation and dissolved organic carbon (DOC) release rates for algae in 30 °/oo sea water.

Carbon fixation rate (mg C g dry wt^{-1} h^{-1})	DOC release rate (mg C g dry wt^{-1} h^{-1})	% DOC
Light incubation:		
6.05	0.13	2.1
6.28	0.21	3.3
6.27	0.31	4.9
6.86	0.20	2.9
7.89	0.27	3.4
9.05	0.15	1.7
9.18	0.57	6.2
mean: 7.37±1.34	0.26±0.15	3.5±1.6
Dark incubation:		
0.020	0.003	15.0
0.010	0.001	10.0
0.008	0.003	37.5
0.013±0.006	0.002±0.001	20.8±14.6

Figure 2. Enteromorpha prolifera: time course of DOC release by algae with a photosynthetic rate of 9.18 mg C g dry wt^{-1} h^{-1}.

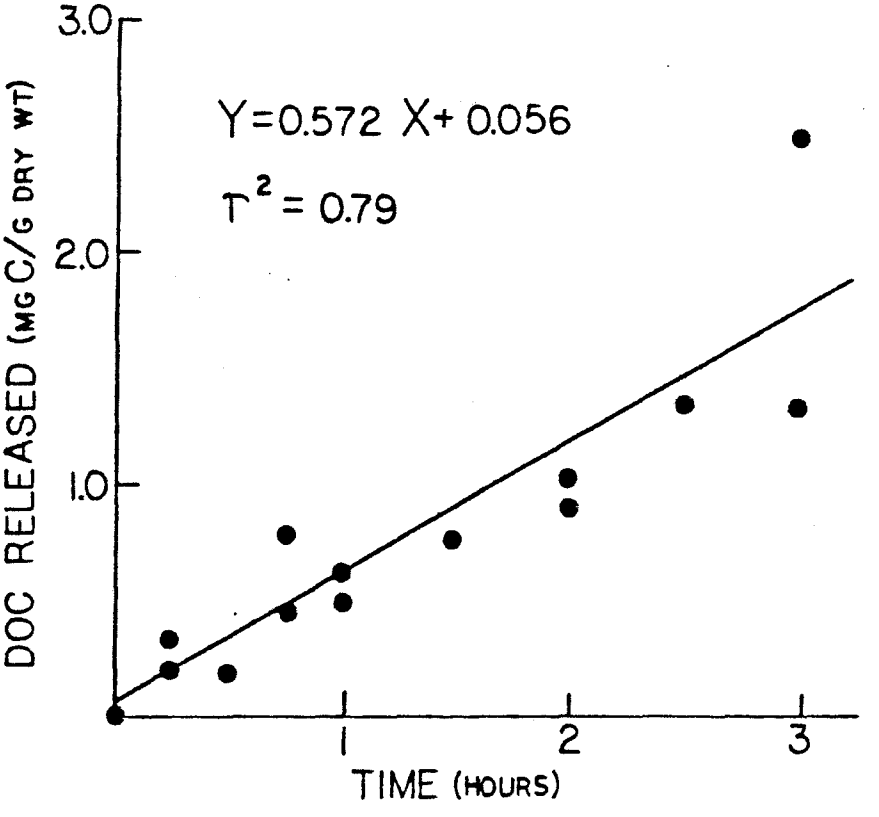
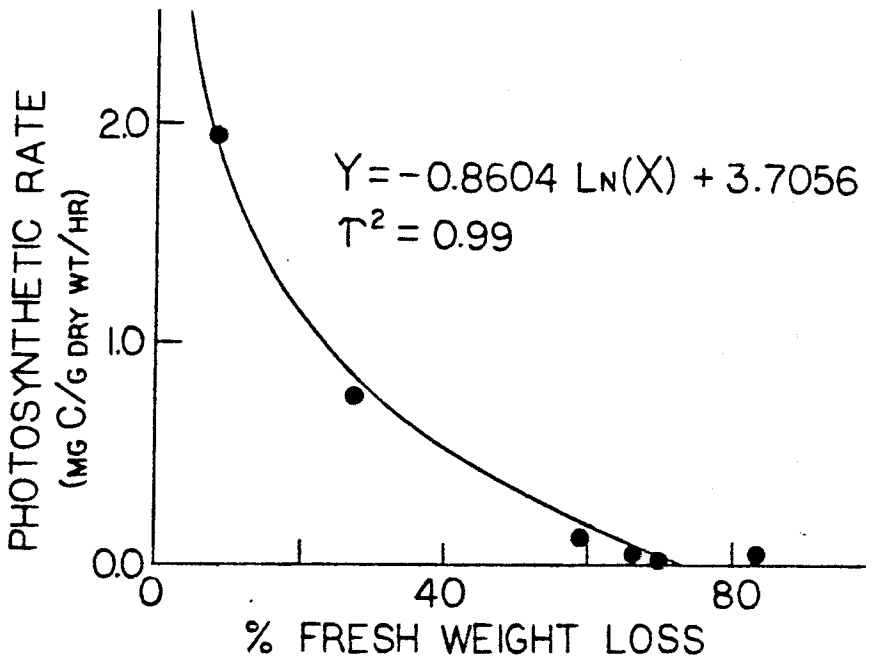

3.0
2.0
1.0
Y=0.572 X+ 0.056
r² = 0.79
DOC RELEASED (MG C/G DRY WT)
1
2
3
TIME (HOURS)

Figure 3. Enteromorpha prolifera: photosynthesis in air by algae as a function of increasing desiccation.


PHOTOSYNTHETIC RATE
(MG C/G DRY WT/HR)
2.0
1.0
0.0
0
40
80
% FRESH WEIGHT LOSS
Y = -0.8604 LN(X) + 3.7056
r² = 0.99

fresh weight loss. However, when these desiccated algae are reimmersed by the incoming tide, they lose some of their recently fixed carbon. The greater the loss of water by algae during exposure, the greater the fraction of fixed carbon that is released by the algae becomes (Figure 4).

Exposed algae may either be submerged by the incoming tide or be subjected to rainfall. Previously labelled algae were desiccated to 65% fresh weight, and carbon fixation determined from a subsample. A portion of the algae was reimmersed in 100 ml of 30 $^{o}/oo$ synthetic sea water, and another was subjected to simulated rainfall: 100 ml of fresh water was sprinkled through an inverted Buchner funnel, and the algae allowed to remain covered by the accumulated water. Water samples were removed at 0, 15, 30, 60, and 180 minutes after initiating reimmersion or rainfall and analyzed for DOC. Release rates were calculated for each time interval between samplings. These slightly desiccated algae release DOC upon reimmersion in a rapid pulse over the first fifteen to thirty minutes (Figure 5). If the reimmersion is due to steady rainfall, the loss of DOC is greater in magnitude and more protracted; initially, the rate of release may be greater than the prior photosynthetic rate.

The carbon fixation rate of Enteromorpha prolifera is only slightly reduced at lower salinities, with the overall density of algae being much more important (see Chapter 4). However, the fraction of fixed carbon that is lost as DOC increases to about 15% in 5 $^{o}/oo$ sea water, from the more typical 5% in 30 $^{o}/oo$ (Figure 6). It is not known whether the DOC release is reduced at higher salinities.

Figure 4. Enteromorpha prolifera: release of previously fixed labelled carbon during one hour following reimmersion of desiccated algae in sea water.

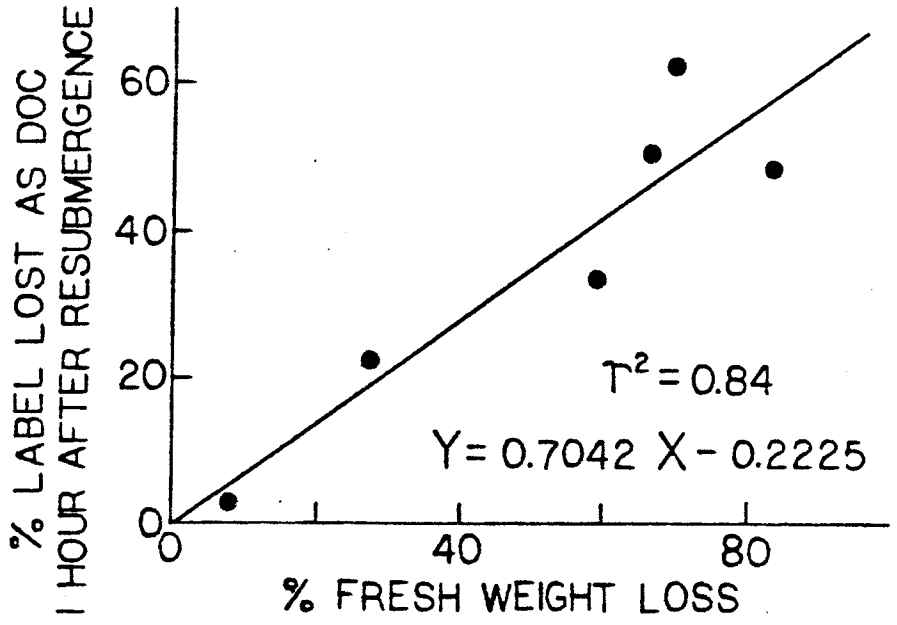

% LABEL LOST AS DOC
1 HOUR AFTER RESUBMERGENCE
60
40
20
0
0
40
80
% FRESH WEIGHT LOSS
r² = 0.84
Y = 0.7042 X - 0.2225

Figure 5. Enteromorpha prolifera: time course for rate of release of previously fixed carbon following reimmersion in sea water or by simulated rainfall.

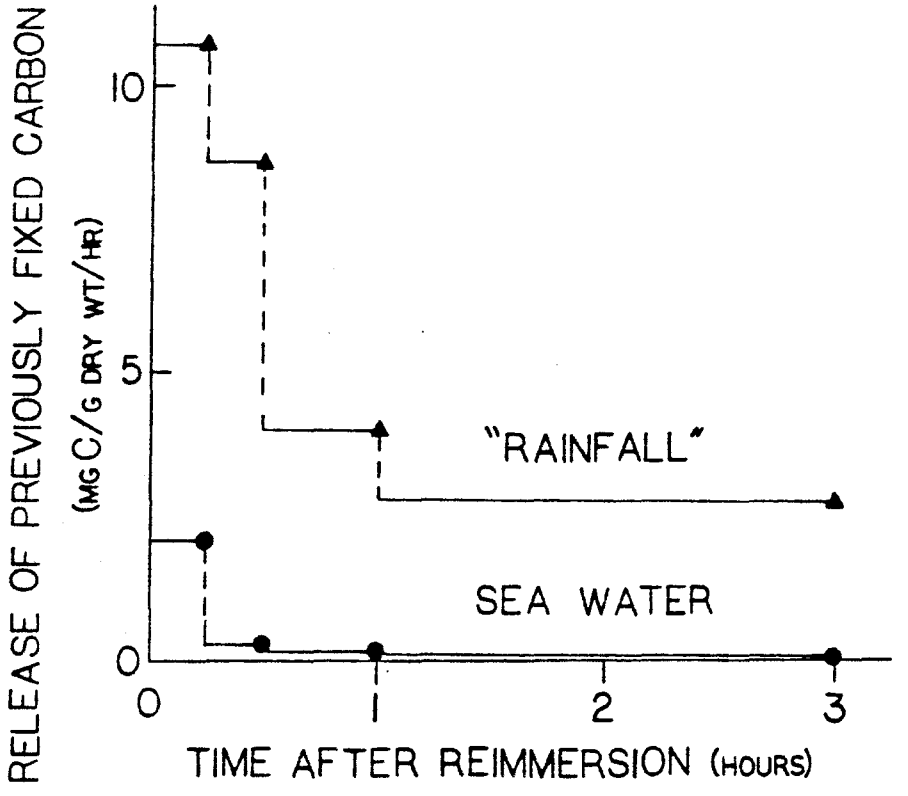
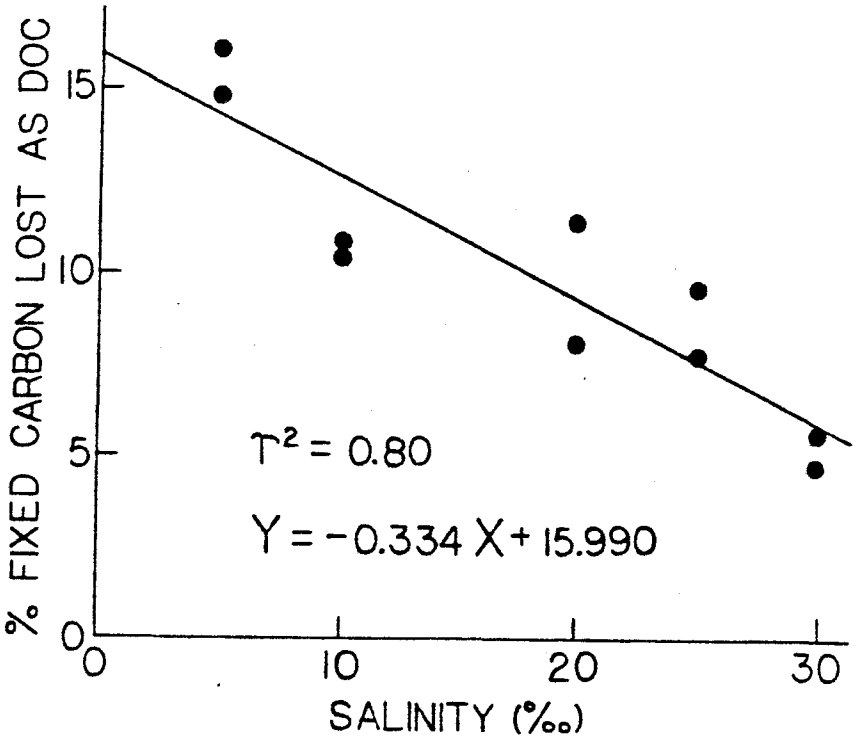

RELEASE OF PREVIOUSLY FIXED CARBON
(MG C/G DRY WT/HR)
10
5
0
"RAINFALL"
SEA WATER
0
1
2
3
TIME AFTER REIMMERSION (HOURS)

Figure 6. Enteromorpha prolifera: percentage of fixed carbon released as DOC for pairs of incubations at five salinities.


% FIXED CARBON LOST AS DOC
15
10
5
0
0
10
20
30
SALINITY (‰)
r² = 0.80
Y = −0.334 X + 15.990

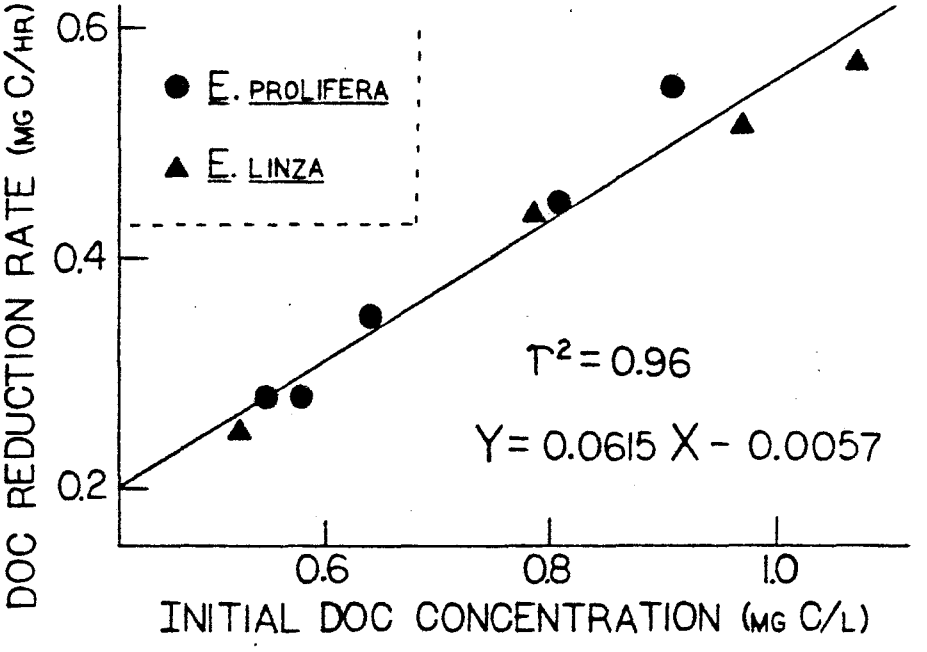
The labelled material that Enteromorpha prolifera releases during short-term photosynthetic incubations probably consists of intermediate metabolites. The heterotrophic microbes from the algal habitat, which are present as epiphytes on the algae and in the sediments, should prove capable of removing labelled DOC from sea water. To test this, 150 ml of water in which algae had been incubating and releasing labelled DOC for three hours were added to replicate foil-wrapped bottles containing either 20 g of mudflat surface sediment or 3.5 g fresh weight of heavily epiphytized E. prolifera. The bottles were held at 10° C for three hours and hand-swirled every 30 minutes to circulate the water without stirring up the sediments. The initial and final DOC concentrations were determined as above. Controls were autoclaved sediments and algae. Table 2 shows the decrease in DOC activity in the presence of these microbes. The control reductions are likely to be due to adhesion of the organic molecules to particulates. Thus, over a three-hour period, about 40% of the available DOC was removed from solution by sediment-associated microbes, and about 47% was utilized by epiphytic microbes.

In a separate experiment, 300 ml of DOC-containing water from incubations with Enteromorpha prolifera and from E. linza were incubated for five hours with sediments. There was a control-corrected decrease of 75.4 ± 5.8% of the DOC from E. prolifera, and a decrease of 71.5 ± 4.1% of the DOC from E. linza. The rate of removal appears to increase directly with the amount of DOC available, and there is no apparent difference in the use of DOC from the two different species of algae (Figure 7).

Table 2. Reduction of DOC activity (dpm = ^{14}C disintegrations per minute) in the presence of microbes. Values are for a three-hour incubation at 16° C.

Initial dpm ml^{-1}	Final dpm ml^{-1}	Decrease	% Decrease	Corrected % (- control %)
Sediments:				
922	795	127	13.8	control
627	282	345	55.0	41.2
942	406	536	56.9	43.1
801	365	436	54.4	40.6
792	385	407	51.4	37.6
690	307	383	55.5	41.7
976	479	497	50.9	37.1
			mean:	40.2 ± 2.3
Epiphytes:				
1193	1126	67	5.6	control
1019	456	563	55.3	49.6
963	542	421	43.7	38.1
1045	532	513	49.1	43.5
917	431	486	53.0	47.4
1100	479	621	56.5	50.8
1245	513	732	58.8	53.2
			mean:	47.1 ± 5.5

Figure 7. Enteromorpha prolifera and E. linza: rate of DOC removal in presence of sediment-associated microbes as a function of initial concentration. Rate is hourly average over the entire five-hour incubation period.


DOC REDUCTION RATE (MG C/HR)
0.6
0.4
0.2
E. PROLIFERA
E. LINZA
$r^2 = 0.96$
Y = 0.0615 X - 0.0057
0.6
0.8
1.0
INITIAL DOC CONCENTRATION (MG C/L)

Discussion

Release of dissolved organic carbon from Enteromorpha prolifera falls among the higher values reported in the literature, particularly for studies that use ^{14}C-incorporation and release techniques (Khailov and Burlakova, 1969; Sieburth, 1969; Moebus and Johnson, 1974; Brylinsky, 1977; Penhale and Smith, 1977); this is largely due to the high photosynthetic rate of this alga. The DOC activity of E. prolifera in this study is composed of recently labelled materials, so there may be other substances being released that have not been detected. Thus, all values reported here are conservative. However, because of the intertidal location of the algae, the duration of submergence in daylight and thus the period of such photosynthesis may be only a few hours at a time, so the values measured here should be realistic.

As compared to my data, the release rates reported by Khailov and Burlakova (1969) and by Sieburth (1969) are higher, presumably due to (1) differences in their methods of measurement, which would detect release of materials synthesized far prior to the time of release, and (2) the potential injury or senescence of their algal material, as they suggest. Moebus and Johnson (1974) found substantial loss of DOC from injured holdfasts of fucoid algae. The potential for overestimation of normal DOC release due to injury of Enteromorpha prolifera in the present study is small, for only the cells at the very ends of the thin filaments would be broken, permitting the incorporation of label into stable products in the incubation medium. The slightly higher release rates for submerged photosynthesis reported by Sieburth (1969) coupled with the lower photosynthetic rates of his brown algae, relative to

E. prolifera, result in his much higher percent release values of 25% compared with about 3.5% in the present study.

The materials most likely to be released during photosynthesis would be relatively small, labile molecules such as amino acids, organic acids, sugars, and sugar phosphates. Patil and Joshi (1970) found a high intracellular turnover of such metabolites in Ulva lactuca, with the ethanol-soluble activity remaining fairly constant over several hours, while the ethanol-soluble components continued to increase in activity. If the pool of potentially releasable molecules is fairly constant in size, one would expect a constant release rate, with a linear accumulation of DOC in the incubation medium.

While some algae, particularly the high-intertidal fucoids, increase their photosynthetic rates in air after some desiccation (Johnson et al., 1974; Quadir et al., 1979), Enteromorpha prolifera shows much reduced carbon fixation rates after as little as 30% fresh weight loss. Such a reduction in photosynthesis is typical for algae with very thin thalli (Imada et al., 1970; Johnson et al., 1974; Wiltens et al., 1978; Quadir et al., 1979).

The observations that (1) increased desiccation of algae results in an increased fraction of fixed carbon lost upon reimmersion and that (2) the release comes in a pulse over the first 15 minutes suggest that moderate desiccation can be quite stressful to the cellular stability of Enteromorpha prolifera. The increased magnitude and duration of DOC release indicate that rainfall on exposed algae is another severe stress. These increases are likely to be due to the influence of reduced salinities as well as the shock of resubmergence.

It is possible that the organics released following reimmersion under stressful conditions include not only the smaller metabolites presumed for typical submergence, but also larger, more complex substances such as polypeptides and structural carbohydrates released by cell wall damage. Such damage could well result in a large but brief pulse release after reimmersion.

Sieburth (1969) found reduced DOC release with lower salinities in fucoid algae and suggested that it was due to lowered photosynthetic rates. The total carbon fixation rate of Enteromorpha prolifera was not substantially reduced with decreased salinity in the present study, and DOC release increased at lower salinities; thus the release is probably passive and may be affected by the osmotic relation between the cells and the surrounding medium.

All of the typical estuarine fluctuations in this study of Enteromorpha prolifera increased the rate of DOC release and/or the fraction of fixed carbon released. This indicates that field populations of the algae are repeatedly confronted with a variety of stresses, and that the fragile structure of these algae (relative to that of the fucoids or laminarians, for example) provides little protection. They are thus able to achieve standing crop levels of 100 to 350 g dry wt m^{-2} over a period of weeks only by virtue of their substantial carbon fixation rates.

The heterotrophic microbes from the algal habitat removed much of the available labelled DOC from the incubation medium over the course of a few hours. If indeed the DOC consists primarily of useful metabolic intermediates, the microbes might scavenge them from the water

by some active transport mechanism against a concentration gradient. Additionally, if the microbes were previously adapted to the availability of the substrates in their habitat, such transport would occur without a lag time for the potential induction of necessary permeases. The observation that the DOC removal rate increases linearly with DOC availability over the range of concentrations measured suggests that such a presumed active transport has not yet reached saturation. However, the result is also consistent with the possibility that DOC removal is due to a passive process such as diffusion of labile molecules into microbial cells, if the molecules are quickly metabolized in some way to maintain the necessary concentration gradient.

CHAPTER THREE

COMPOSITION OF RELEASED ORGANIC CARBON

Enteromorpha prolifera releases recently fixed organic carbon into the surrounding medium. Under conditions observed in an estuary, from 3 to 15% of initial photosynthate is lost by these algae during photosynthesis. The major limitation of the technique employed in this study for quantifying DOC release is that the short-term observations do not permit measurement of the release of materials that have resided in the algal tissues for longer than the durations of the experimental incubation.

In Ulva lactuca, which should be similar to Enteromorpha spp., low-molecular-weight, intracellular metabolites have high turnover rates (Patil and Joshi, 1970, 1971); that is, radioactive label rapidly passes through these pools of small molecules and into larger, more complex substances. Other investigators (e.g., Wetzel and Manny, 1972; Fogg, 1976; Sondergaard, 1981) have found that such labile metabolites comprise the majority of fixed carbon released by aquatic macrophytes. Under the experimental conditions used in the present study, most of the released DOC that is detected should also consist of small organic molecules.

The purposes of this portion of the present study were to determine the approximate size of ^{14}C-labelled DOC from Enteromorpha prolifera by gel fractionation, and to relate the observed size pattern to the conditions under which the DOC was released and to the patterns of labelled materials inside the algal tissue.

Materials and Methods

Preparation of DOC

Enteromorpha prolifera was collected from the field study site during the months of August to October in 1982 and held as described in Chapter Two. Photosynthetic incubations were performed in the 1.5-liter Plexiglas chambers to obtain sufficient quantities of DOC-containing water for replicate analysis. At predetermined intervals during incubations, 150-ml water samples were removed from the chambers and acidified to pH 2.5 with 5.0 N HCl. The water was flushed for 20 minutes with CO_2-free air to drive off inorganic label and then adjusted to neutral pH with 5.0 N NaOH.

A 75-ml subsample was then concentrated to 5 ml. The sample was placed in a side-arm flask in a 65° C water bath, and the vapor pressure was reduced by aspiration to enhance evaporation. Often during this procedure, salt crystals would form on the sides of the evaporation flask. The residues were washed down with distilled water, and the increased fluid volume was reconcentrated to 5.0 ml.

Gel Fractionation

Labelled compounds in the DOC concentrate were separated by Sephadex G-15 (Pharmacia Fine Chemicals) gel fractionation. This procedure resolves compounds with molecular weights less than about 1500. Larger substances elute in the void volume, for they are excluded from the pore space of the gel beads. Smaller molecules, which can enter the pore spaces, are retarded during elution owing to the greater

relative volume in which they move. A column with a bed height of 52 cm and an inner diameter of 1.9 cm was prepared with 50 g of Sephadex G-15 that was swollen and then packed in the column with pH 7.4 phosphate buffer. The elution characteristics of the column were calibrated with bovine hemoglobin (void volume), ^{14}C-lactose (disaccharide standard), ^{14}C-galactose (monosaccharide standard), ^{3}H-leucine (amino acid standard), and NaCl (total elution volume). An elution rate of 0.85 ml minute^{-1} was maintained with pH 7.4 phosphate buffer in all fractionations.

Half of a 5.0-ml DOC concentrate prepared as described above was layered at the surface of the gel bed; the high salt concentration from the evaporation procedure proved useful in preventing mixing of the sample with the surrounding buffer. Aliquots of 5.0 ml were collected in storage vials during elution, and ^{14}C-activity in 2.5-ml subsamples of these aliquots was determined by liquid scintillation counting.

Serial Extraction of Algal Tissues

Algae were extracted to determine the allocation of recently fixed, labelled carbon within actively photosynthesizing thalli. The extraction procedure of Josselyn (1978) served as a guide. Photosynthetic incubations were performed in 30 $^{o}/_{oo}$ synthetic sea water as described in Chapter Two. After 1, 2, and 3 hours, labelled algae were removed from the 1.5-liter chambers and frozen in foil at -5° C to stop metabolism without chemically disrupting the cells. The algae were then dried at 90° C and ground to a fine powder.

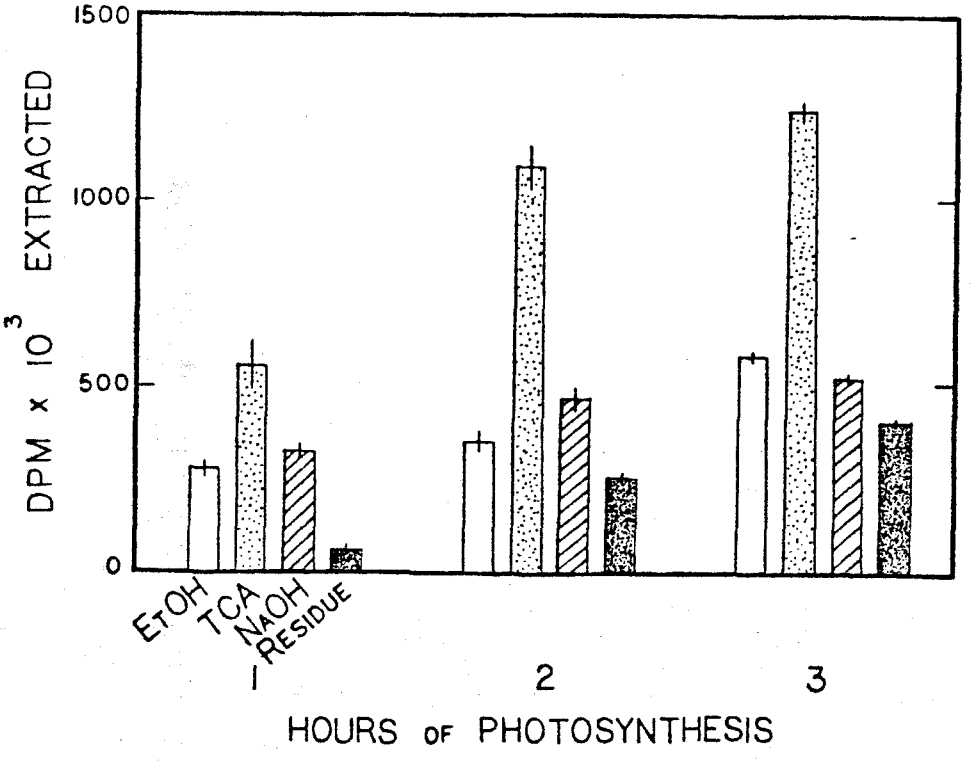
Replicate aliquots of ground algae were extracted for 10 minutes with hot 90% ethanol, then centrifuged for 10 minutes to pellet the nonextracted tissues. The ethanol was decanted, the pellet dried, and the weight lost by extraction determined. This procedure was repeated using 10 ml of hot 5% trichloroacetic acid (TCA) and hot 1.0 N NaOH. The initial ethanol extract should contain small, labile metabolites and the photosynthetic pigments. The TCA should precipitate proteins and solubilize polysaccharides and nucleic acids. The NaOH extract should contain the proteins, and the final residue should consist of structural components from the cell walls, such as hemicelluloses.

The activities of subsamples of the extracts were determined by liquid scintillation, as were the activities of the pellets. Thus it is possible to follow the accumulation of ^{14}C in the various biochemical constintuents through time, and to determine specific activities for each constituent on a weight basis.

Results

Labelled carbon is present in all general biochemical fractions after one hour of photosynthesis, and the amount of label in each fraction increases through the three hours of algal incubation (Figure 8). After one hour, 23% of labelled carbon occurs in ethanol-soluble substances, with the remainder present in stable metabolic end-products. As the duration of photosynthesis increases, the relative amount of ^{14}C-activity present in labile metabolites and pigments decreases only very slightly, to 21%, while allocation of recently fixed materials increases in the structural components in the residue from 5 % after

Figure 8. Enteromorpha prolifera: accumulation of ^{14}C-activity in serial extracts of dried algae after photosynthesis. Error bars are standard deviations of four replicate measurements.


1500
1000
500
0
DPM x 10^3 EXTRACTED
ETOH
TCA
NAOH
RESIDUE
1
2
3
HOURS OF PHOTOSYNTHESIS

one hour to 14% after three hours. The amount of label fixed in proteins decreases relative to other fractions. The weight-based specific activity also increases through time for each fraction except the ethanol extract, which appears to decrease slightly (Figure 9). While the relative weights of the TCA, NaOH, and residue fractions remained quite similar for the three algal incubations, the weight of the ethanol-extractable material increased slightly at three hours; this caused the observed decrease in specific activity.

A 5.0-ml subsample of the ethanol extract from each incubation was evaporated to dryness, and the nonvolatile residue redissolved in 5.0 ml distilled water. Pigments did not go into solution and remained as a visible film on the walls of the glass vial. While it is possible that other, noncolored substances did not go into solution, a mean of 85% of ethanol-soluble activity was also water soluble in these experiments. However, there was a consistent decrease in the water-soluble fraction of total ethanol-soluble activity through the three hours; it is possible that this indicates increased labelling of pigments through the duration of the incubations. If this is the case, it may help explain the decrease in specific activity observed in Figure 9.

Salt was added to increase the density of 2.5 ml of a sample of such an aqueous-soluble fraction from an ethanol extract; its components were then separated by gel fractionation. The elution pattern of ^{14}C-activity changes through time, as does the total activity in the samples (Figure 10). The increasing peak at an elution volume of about 105 ml corresponds to the monosaccharide standard, and the peak at about 95 ml corresponds to the disaccharide. There are significant amounts of

Figure 9. Enteromorpha prolifera: hourly changes in weight-based ^{14}C specific activities in serial extracts of dried algae after photosynthesis. Error bars are standard deviations of four replicate measurements.

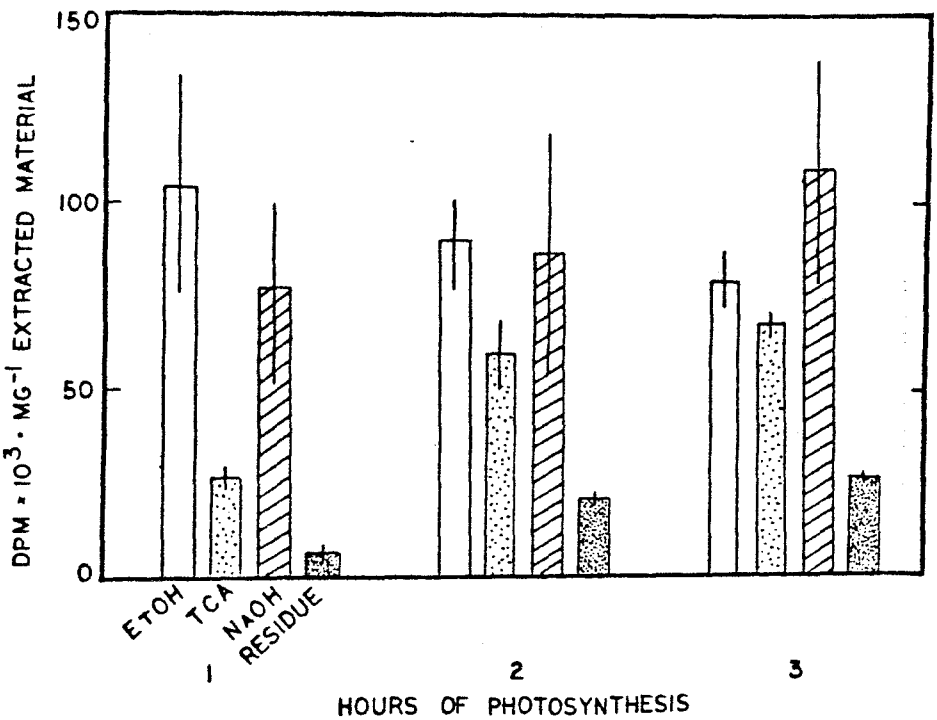
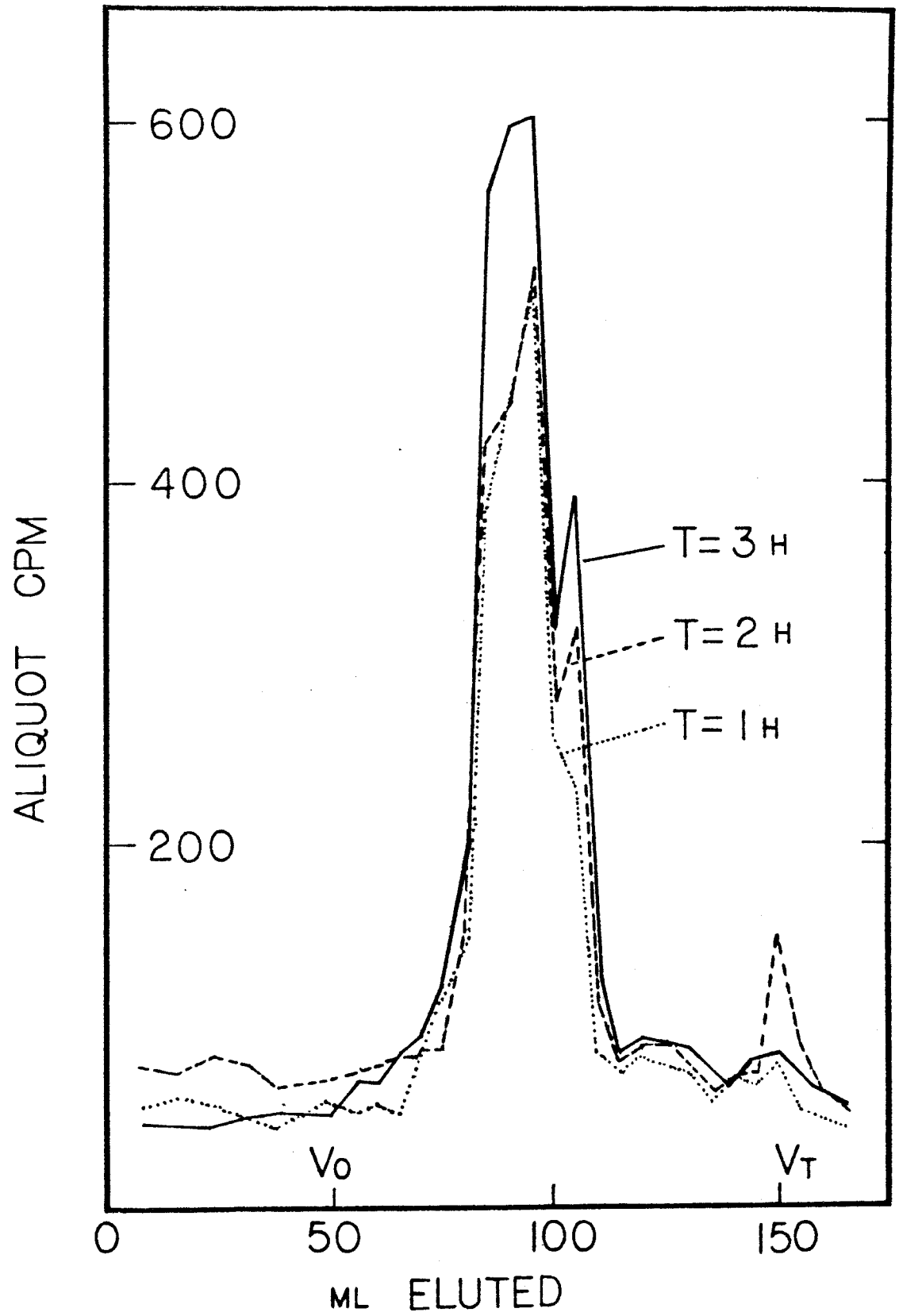

150
100
50
0
DPM × 10³ · MG⁻¹ EXTRACTED MATERIAL
ETOH
TCA
NAOH
RESIDUE
1
2
3
HOURS OF PHOTOSYNTHESIS

Figure 10. Enteromorpha prolifera: Sephadex G-15 elution patterns of aqueous-soluble components in ethanol extracts of dried algae after photosynthesis. Total ^{14}C activity per 5-ml aliquot is indicated as counts per minute (CPM). V_o indicates the void volume of the column, and V_t the total elution volume.


600
400
200
ALIQUOT CPM
T= 3 H
T= 2 H
T= 1 H
Vo
VT
0
50
100
150
ML ELUTED

labelled substances that are larger than the disaccharides (eluted earlier), but none larger than a molecular weight of about 1500, for no activity appears in the void volume. The lesser amounts of substances that are smaller than the monosaccharide standard (eluted later) should include amino and organic acids.

Gel fractionation of DOC concentrates from one- and three-hour photosynthetic incubations indicate that all of the DOC does indeed consist of small molecules (Figure 11). An increasing peak corresponds to the monosaccharide standard at about 105 ml, and a large amount of smaller substances probably consist of amino and organic acids. A comparison of the elution patterns of 3-hour DOC and 3-hour ethanol extract shows that only the smallest substances from the intracellular metabolites are being released (Figure 12).

Three-hour algal incubations were performed in 30 $^{o}/oo$ and 2 $^{o}/oo$ synthetic sea water as in Chapter Two. Water samples were removed from each, and DOC concentrates prepared as described above. The total ^{14}C-activity in DOC released during the 2 $^{o}/oo$ incubation (2 ppt DOC) is greater than that from the 30 $^{o}/oo$ incubation (30 ppt DOC), in agreement with the trend in Figure 6. The gel fractionation elution patterns are similar (Figure 13), with most of the activity occurring in those aliquots that correspond to the monosaccharide standard. There appears to be a greater variety of labelled substances in the 2 ppt DOC that are smaller than the monosaccharide, as compared with the 30 ppt DOC, but the resolution of these substances may simply be due to their greater activity over background rather than their release at low salinity and absence at higher salinity.

Figure 11. Enteromorpha prolifera: Sephadex G-15 elution patterns of DOC concentrates from one-hour and three-hour photosynthetic incubations in 30 °/oo synthetic sea water.

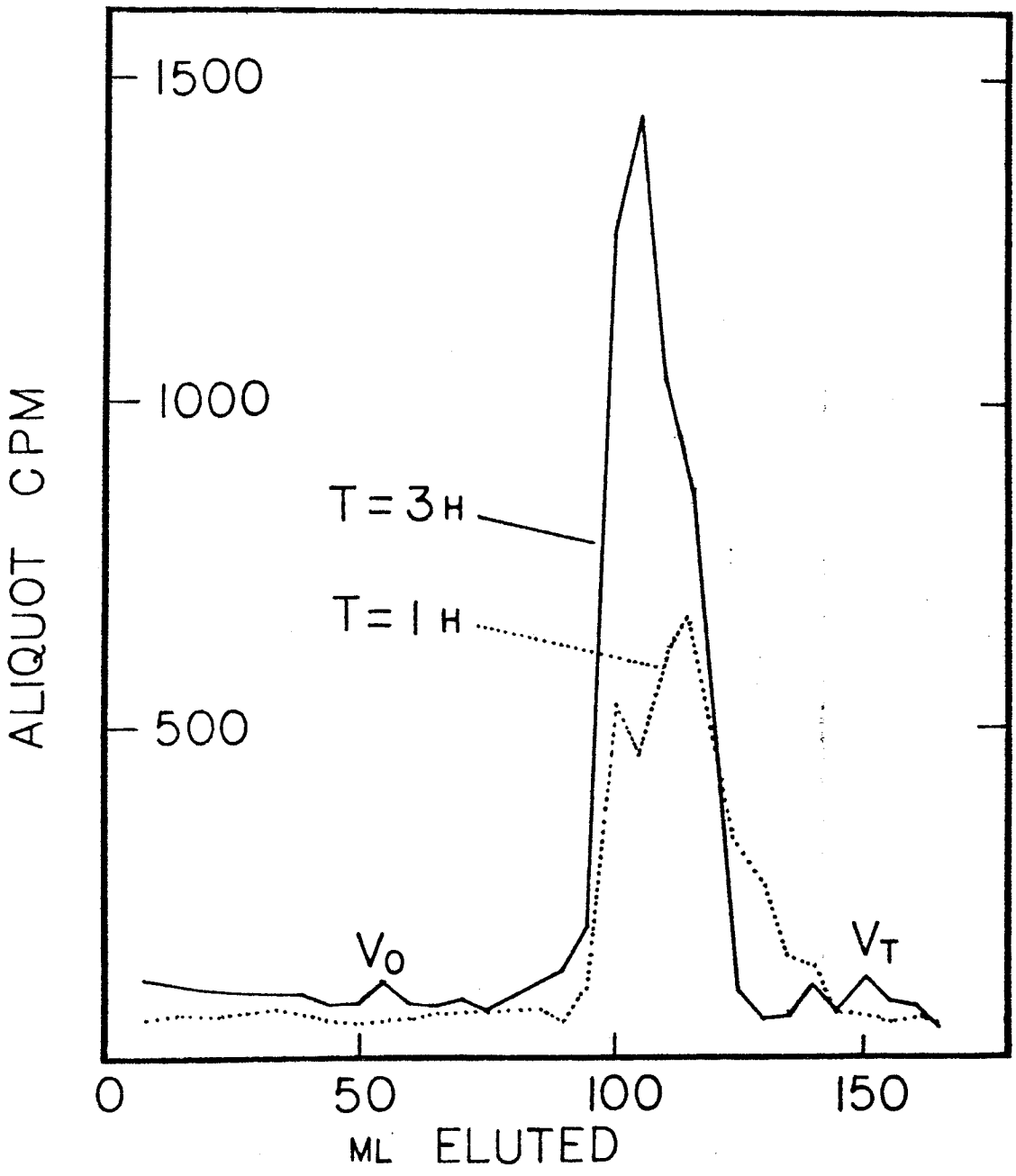

1500
1000
500
ALIQUOT CPM
T=3H
T=1H
V_O
V_T
0
50
100
150
ML ELUTED

Figure 12. Enteromorpha prolifera: comparison of Sephadex G-15 elution patterns of DOC concentrate and of aqueous-soluble components in ethanol extract of dried algae from three-hour photosynthetic incubation.

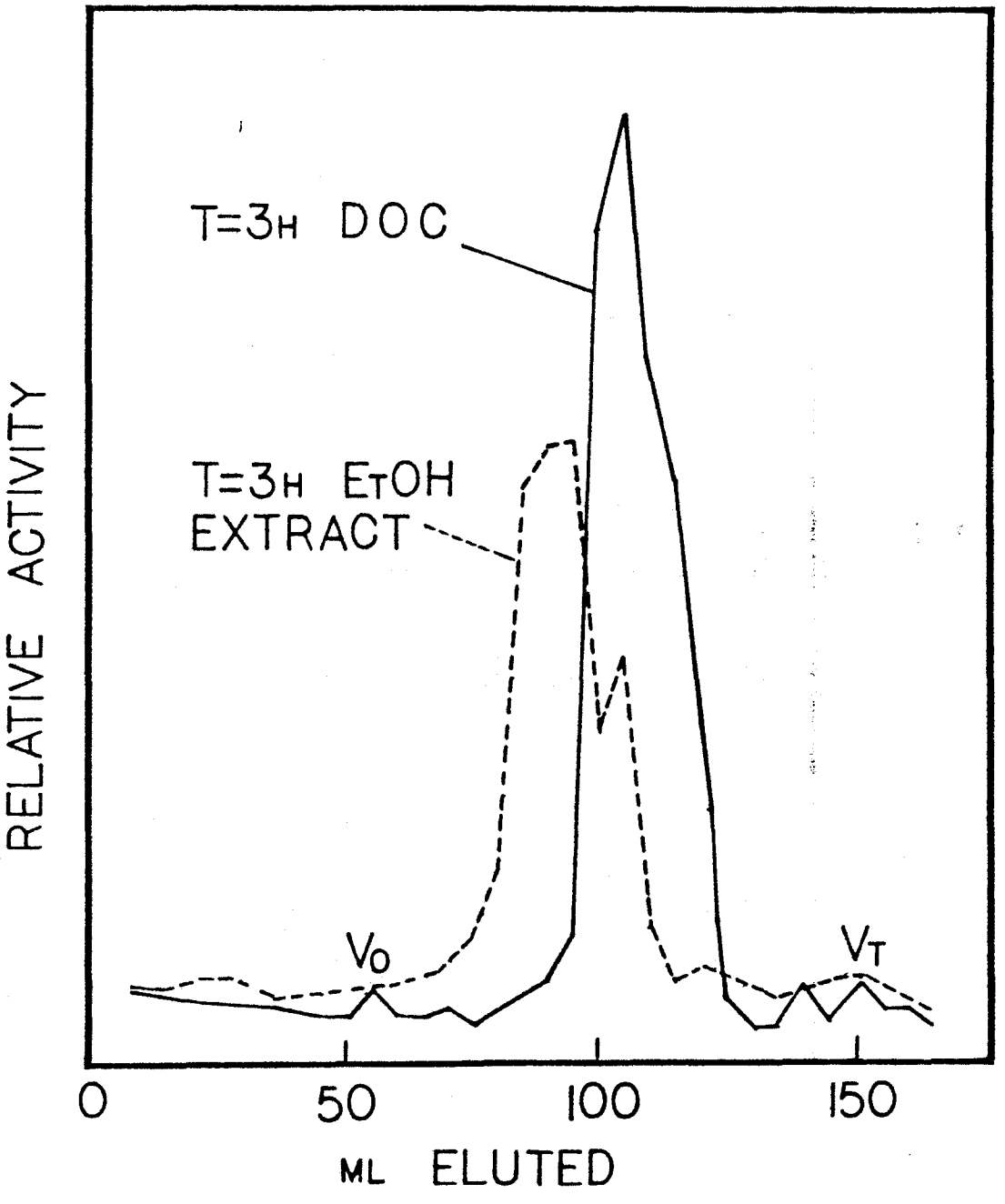
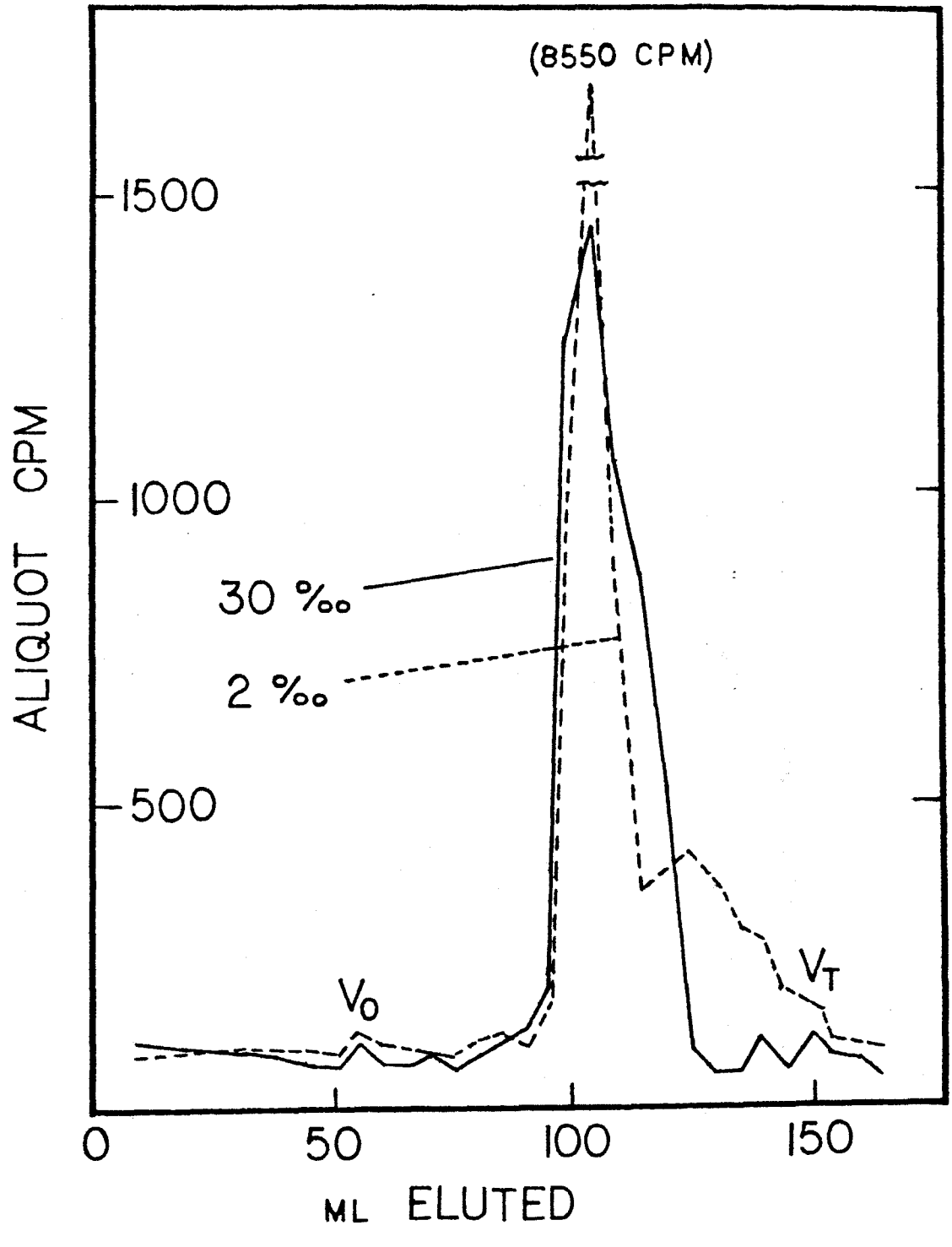

T=3H DOC
T=3H ETOH EXTRACT
RELATIVE ACTIVITY
VO
VT
0
50
100
150
ML ELUTED

Figure 13. Enteromorpha prolifera: comparison of Sephadex G-15 elution patterns of DOC concentrates from three-hour photosynthetic incubations in 30 °/oo and 2 °/oo synthetic sea water.


(8550 CPM)
1500
1000
500
ALIQUOT CPM
30 ‰
2 ‰
V_0
V_T
0
50
100
150
ML ELUTED

Recall that tidal reimmersion of slightly desiccated algae and rainfall upon exposed algae both cuase a brief pulse release of DOC (Figure 5). A DOC concentrate was prepared from simulated rainfall upon previously labelled algae (see Chapter Two). Gel fractionation of this DOC indicates the presence of 15% of the total ^{14}C-activity in the void volume, which contains substances with moleculare weights larger than about 1500 (Figure 14). We presume from the weight-based specific activities in Figure 9 that this activity represents a proportion of fixed carbon roughly equivalent to that present in smaller substances. The elution pattern of the remainder of the activity more closely resembles that of an ethanol tissue extract than of DOC released during normal photosynthesis.

It was demonstrated in Chapter Two that estuarine heterotrophic microbes can rapidly utilize DOC from Enteromorpha. Gel fractionation of DOC concentrates from the beginning and the end of a 3-hour sediment-associated microbial incubation show the removal of much of the labelled DOC from the water (Figure 15). A slightly greater proportion of the very small metabolites were removed by the microbes.

Discussion

In their studies of Ulva lactuca, Patil and Joshi (1970, 1971) found a slight decrease in the proportion of label present in low-molecular-weight metabolic intermediates as the duration of photosynthesis increased. They also noted fixation of label into stable substances after incubations as short as 5 minutes. Percival and Smestad (1972) found similar ^{14}C-fixation patterns in their work with Ulva

Figure 14. Enteromorpha prolifera: comparison of Sephadex G-15 elution patterns of DOC concentrate from simulated rainfall on previously labelled algae, of DOC concentrate from three-hour photosynthetic incubation, and of aqueous-soluble components from ethanol extract of dried algae after three hours of labelling. The origins of the elution curves have been displaced vertically to facilitate comparison.

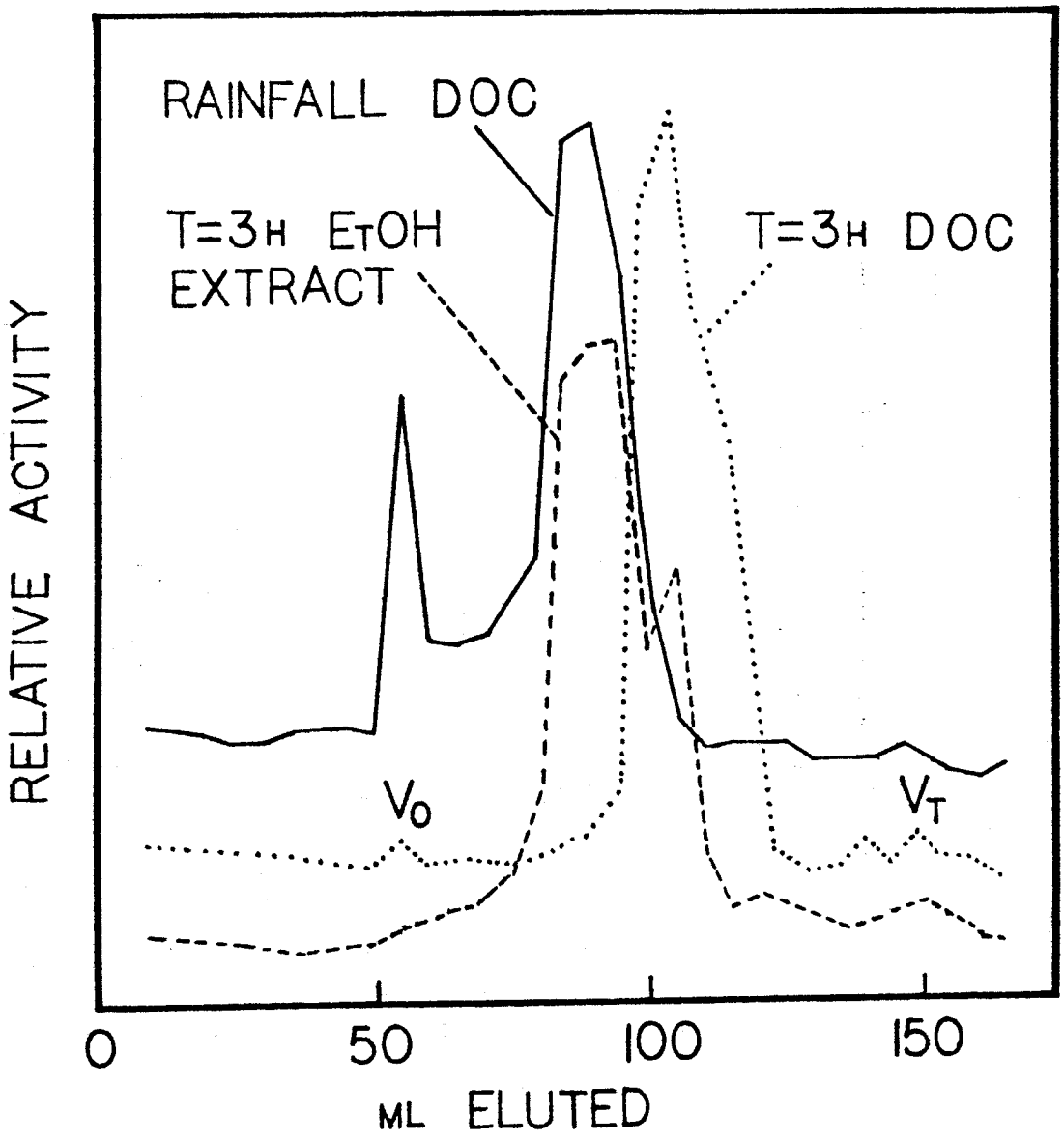
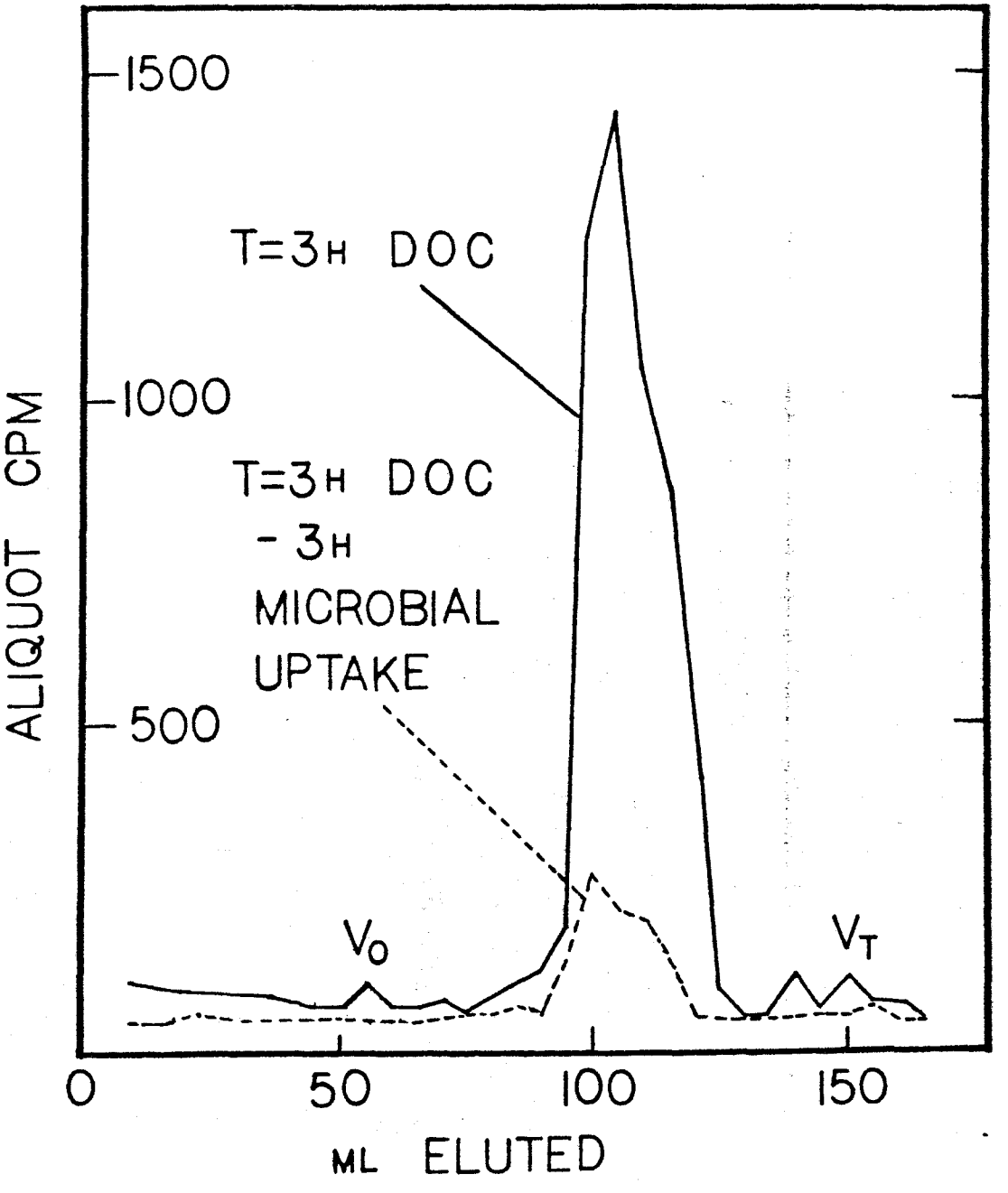

RAINFALL DOC
T=3H ETOH EXTRACT
T=3H DOC
RELATIVE ACTIVITY
V_0
V_T
0
50
100
150
ML ELUTED

Figure 15. Enteromorpha prolifera: comparison of Sephadex G-15 elution patterns of DOC concentrates from three-hour algal photosynthesis and after three additional hours in the presence of sediment-associated microbes.


1500
1000
500
ALIQUOT CPM
T=3H DOC
T=3H DOC
- 3H
MICROBIAL
UPTAKE
V_O
V_T
0
50
100
150
ML ELUTED

lactuca; they noted more label occurring in stable products than in ethanol-soluble substances after only 10 minutes of photosynthesis. Li and Platt (1982) found a very small proportion of label in metabolites after 2-hour photosynthetic incubations with phytoplankton. The pattern in Enteromorpha prolifera from the present study indicates both rapid carbon fixation and increasing allocation of new photosynthate to structural materials and possibly pigments.

The gel fractionation of water-soluble substances present in the ethanol extract shows very small amounts of label in those aliquots that should contain amino acids and organic acids. Patil and Joshi (1970) found most label in amino acids within the ethanol extract of Ulva after one hour of photosynthesis. It is possible that the extraction procedure used in the present study underestimates the amounts of these substances in algal tissues. The lag time between removal of algae from labelled media and the extraction with hot ethanol may permit further metabolism of labile constituents in spite of efforts to prevent this by freezing the algae. This may account for the reduced proportion of label in such small metabolites in the ethanol-extract elution pattern as compared with the DOC elution pattern. It is unlikely that amino and organic acids would have been released in quantity as DOC that were not also sufficiently water soluble to appear in the ethanol-extract aqueous gel fractionation. Nonetheless, there are labile, intracellular components that are not being released as DOC.

Even though large, stable molecules appear within tissues of Enteromorpha within the first hour of photosynthesis, only the small

metabolites are released as DOC. Thus, the short-term incubations do not greatly underestimate total DOC release, unless large substances are only released through long-term turnover of cell membrane or wall materials. Wetzel and Manny (1972), without the use of radioisotopes, found that about 90% of the organic matter released by Najas flexilis, a freshwater macrophyte, is composed of sugars and other labile, low-molecular-weight substances. Fogg (1976) measured the release of glycollate from some photosynthesizing tropical marine macrophytes. Sondergaard (1981) determined that most of the ^{14}C-labelled DOC released by the freshwater plant Littorella uniflora has a molecular weight of about 200. Nalewajko and Lean (1972) found that some freshwater phytoplankton release small metabolites. Saks (1982) observed release of amino acids from the endosymbiotic alga Chlamydomonas provasolii when in axenic culture.

It is possible that not all of the small cellular metabolites are available for release. Wheeler and Stephens (1977) found that some free amino acids were isolated from general metabolism in the microchlorophyte Platymonas. They were unable to determine the mechanism of segregation of these amino acids. Mague et al. (1980) found that release of labelled materials by phytoplankton could continue during periods without further incorporation of label, and suggested that this was due to mobilization of larger end-products (especially polysaccharides) that maintained the intracellular pools of small, labile substances from which the extracellular products were derived. They noted that only a subset of the labelled intracellular amino acids were released.

Reduced salinity may increase the variety as well as the quantity of released substances in Enteromorpha prolifera. The small substances which were resolved in elution of 2 ppt DOC may be organic solutes synthesized in response to reduced salinity (Kirst and Bisson, 1979). Vogel et al. (1978) observed increased release of amino acids and sugars from microalgae under filtration pressure, but they did not find substances being released during filtration that were not also released during photosynthesis. The increased pressure potential of an algal cell due to reduced external salinity may be partially responsible for the increased DOC release, as the increased vacuum pressure from filtering cells is considered to be responsible for higher DOC release in phytoplankton studies. If this comparison is valid, it would strengthen the idea that DOC release from Enteromorpha is a passive process that may be mediated by the osmotic potential of the cells.

Rainfall on exposed algae causes the release of a wide spectrum of substances. Sieburth (1969) found increased release of DOC with both light and heavy simulated rainfall and with a light natural rainfall on a variety of emersed algae. The loss of materials with higher molecular weights than 1500 from Enteromorpha prolifera suggests that cell lysis may have occurred. However, most of the label released during rainfall shows an elution pattern more closely resembling that of cellular metabolites. Thus, while rainfall may not cause widespread cell lysis, it apparently stresses the cell walls and membranes to a considerable degree. Even though Enteromorpha spp. are among the most euryhaline known, with a remarkable tolerance to lowered salinities (e.g., Black, 1971; Reed and Russell, 1978, 1979), the sudden reduction

in salinity that occurs when rain falls on an exposed intertidal area is quite stressful to these algae.

Sediment-associated microbes removed much of the DOC from solution over the course of a few hours as indicated in Chapter Two. When marine and presumably estuarine microbes are presented with an increase in free amino acids, they readily take them out of solution, even if nitrogen is not limiting at the time (Williams and Gray, 1970). Luxury consumption of nitrogen (uptake of nitrogen in excess of what is necessary for immediate metabolic needs) would be quite advantageous for microbes that live in frequently nitrogen-poor habitats. The elution patterns from the microbial incubations in the present study indicate that a greater proportion of labelled substances that elute with the amino acid standards were removed from solution in three hours. These substances are not necessarily amino acids, but their quantity, elution pattern, and preference by microbes all strongly suggest that they are. Much of the DOC corresponding to the monosaccharide standard was also removed. Heterotrophic microbes in many aquatic habitats have been found to assimilate and respire these types of metabolites (Nalewajko and Lean, 1972; Bauld and Brock, 1974; Williams and Yentsch, 1976).

Thus the DOC that Enteromorpha prolifera releases during photosynthesis consists primarily of monosaccharides with lesser amounts of disaccaride and some smaller substances. The short photosynthetic incubations may not underestimate DOC release, for even though label is present in larger metabolic endproducts after a short time, only the small metabolites are released. Reduced estuarine salinity appears to be a slight stress to photosynthesizing algae, but rainfall is a

severe stress to emersed algae. As expected, microbes from mudflat sediments can remove released algal metabolites from solution, and appear to prefer the smaller substances that are released.

CHAPTER FOUR

PRIMARY PRODUCTION BY GREEN ALGAL MATS

The potential importance of Enteromorpha spp. mats in the Coos Bay estuary was proposed in Chapter One. Productivity by green macroalgae has only recently been examined. The appearance of large populations is sporadic; factors that control the rapid growth and the just-as-rapid disappearance of the algae may include light limitation (Kier and Todd, 1967; Shellum and Josselyn, 1982), grazing by small herbivores (Price and Hylleberg, 1982; Warwick et al., 1982), and physical removal by wave action (Fitzgerald, 1978).

While benthic algae typically do not accumulate biomass to the standing crop levels that are characteristic of seagrasses or salt marshes, their annual production may be quite substantial through high material turnover (e.g., Mann, 1972; Brinkhuis, 1977; Hatcher et al., 1977). Macroalgae may grow at a rate just sufficient to replace distal frond tissues that are lost by erosion and fragmentation, or to replace individuals lost by grazing and wave removal.

Annual production of macroalgae can be estimated in several ways (Brinkhuis, 1977). If it is assumed that there is little or no loss of living material from the field population, total production should be equivalent to the maximum level of standing crop that is attained during the growing season. Secondly, the growth of marked individuals can be observed through time and extrapolated to the age and size distribution of the population as a whole. A third technique for estimating total production involves the measurement of photosynthesis

by algae under a range of conditions that reflect actual field states, and extrapolation of these production rates within limits dictated by field parameters.

The shortcomings of using maximum biomass as a measure of total production have already been pointed out. The fragile filaments and sheets of those species encountered in the Coos Bay green algal mats are certainly subject to losses during the growing season; indeed, great rafts of drift algae are seen floating about the bay in summer and fall. Therefore, the maximum standing crop of these algal mats will not reflect actual production. The second method of determining production, that of measuring the growth of marked individuals (Mann, 1972), is also likely to provide a poor estimate for Enteromorpha spp. There are considerable procedural difficulties in measuring the actual growth of thin, filamentous, branching algae. Additionally, broken thalli may regenerate, or reversing tidal currents may intertwine several individuals. These larger masses will function as a single individual through effects of self shading, resistance to currents, susceptibility to grazing, etc.

Therefore, I decided to estimate total algal mat production by extrapolation of photosynthetic rates. Several types of information are required by this approach. First, the photosynthetic responses of the algae under conditions observed to occur in the estuary must be measured. Factors that influence photosynthesis must be identified, their range of values in the field determined, and the functional responses of algae under various combinations of the variable must be measured in some way that will permit multivariate predictions to be

made. For estuarine algae, the factors of greatest importance will be light intensity (e.g., King and Schramm, 1976; Arnold and Murray, 1980), temperature (Yokohama, 1973; Shellum and Josselyn, 1982), desiccation of exposed intertidal algae (Johnson et al., 1974; Quadir et al., 1979), and algal density (Littler, 1979; Littler and Arnold, 1980).

Secondly, the amount of time available for net production by algae must be determined. Clearly, the initial limit for this component is daylength. However, those factors that reduce photosynthesis (low light intensity, increasing desiccation) must also be considered in computing the duration of net production. These factors will likely vary with intertidal elevation; therefore, the position of the algae must be determined and incorporated into estimation of total production.

Thirdly, the photosynthetic biomass of the population must be estimated. While many algae have separate tissues for photosynthesis versus structural support and reproduction, Enteromorpha and Ulva spp. are completely photosynthetic. Thus, potentially all of the biomass measured in the field can contribute towards production. However, increasing standing crop may limit carbon fixation through effects of self shading (Bach and Josselyn, 1978; Gordon et al., 1980), competition for inorganic carbon or other nutrients, and reduced boundary layer disruption (Westlake, 1967). Alternatively, increased standing crop may moderate desiccation and permit sustained photosynthesis by emersed algae (Kanwisher, 1957). While field surveys of standing crop provide information on algal density at precise intervals, a distinct character of the green algal mats being studied is their rapid growth and disappearance. Therefore, the photosynthetic biomass should be

adjusted in some fashion between field censuses to account for the changing abundance.

The purposes of this section of the present study were to estimate total production of a green algal mat with the approach delineated above, to compare the production estimate with observed changes in standing crop, and to estimate how the total algal production becomes available to the estuarine system in various forms.

Methods and Materials

Algal Standing Crop

In order to follow the species composition, spatial distribution, and abundance of an algal mat through time, permanent sampling levels were fixed in the field study site in South Slough (Figure 1). Stakes were placed at five elevations at 1.0-foot (0.3-meter) intervals between MLLW (0.0 feet) and 4.0 feet (1.2 meters). Elevations were determined with a stadia rod and sighting level and referenced against the tide staff in the nearby Charleston Boat Basin at several slack tides.

During the growing season of April through October, algae were collected on the best spring low tide series of each month. Fewer collections were made during the winter. At each elevation studied, five samples, with three subsamples each, were taken at preselected random distances (Table VI in Huntsberger and Billingsley, 1977) along a horizontal transect in four separate areas. Thus, each month's survey contained collections of (5 elevations) x (4 areas per elevation) x (5 samples per area) x (3 subsamples per sample) = 300 subsamples.

Each subsample consisted of all algae that occurred within the area covered by an inverted 100 x 15 mm petri dish.

Collected algae were returned to the lab, where they were washed free of sediments and small grazers, separated by species, and weighed. Initially, both fresh weight (gently blotted of excess water) and dry weight (after overnight drying in a 90° C oven) were measured. After the first year of sampling, only dry weight was measured.

Exposed Production

Total exposed production will be some multiplicative function of the duration of daylight exposure, the biomass of the photosynthetic population, and the rate of carbon fixation by emersed algae.

Daylight Exposure

The time available for photosynthesis by exposed algae was estimated with a computer program that simulates tide curves and daylength changes. The program computed daily mean values of total daylight exposure for each month at the five intertidal elevations at which standing crop data were collected. This program and the general implications of tidal patterns in the Pacific Northwest are discussed further in the Appendix.

Photosynthetic Biomass

It is possible that not all of the algae within an exposed, compressed mat receive sufficient light to carry on net photosynthesis (Bach and Josselyn, 1978; Gordon et al., 1980). In order to determine

light penetration into an algal mat, increasing amounts of fresh algae were placed over one receptor cell of a two-cell submarine photometer (Kahl Scientific Instrument Corporation, Model 269WA310, internally corrected for photosynthetically active radiation, PAR, wavelengths between 400 and 700 nm). The fraction of available light (control cell) that passed through the tangled algae was measured.

The portion of the exposed algal mat that exhibits net photosynthesis may be estimated by combining the light-attenuation function, average surface light intensities for each month, and photosynthesis-light intensity functions (P-I curves), which indicate the light intensities at which photosynthetic saturation and compensation occur (e.g., King and Schramm, 1976; Arnold and Murray, 1980). Only that amount of algae estimated to show net photosynthesis was used as the effective standing crop or photosynthetic biomass in production predictions.

Exposed Photosynthesis

Exposed algae may lose increasing amounts of internal water by evaporation during daylight hours. In order to determine desiccation of exposed algae, variable quantities of fresh algae were placed in 100 x 15 mm petri dishes with a known weight of water-saturated sediment covering the bottom. The open dishes were positioned in a random grid and placed outside in late morning. At time intervals up to a total desiccation duration of three hours, the total weights of algae, sediments and dish, and of just sediments and dish were measured. Dry weights of algae and sediments were determined after overnight drying in a 90° C oven.

If it is assumed that the algal mat is drying out during the exposed daylight period, the changing photosynthetic rate may be estimated by predicting the degree of desiccation of the entire algal mat at hourly intervals, and then applying the photosynthesis function for drying algae determined in Chapter Two (Figure 3).

Receding tides may leave an algal mat resting in a thin layer of water that has been trapped on the mudflat surface by small topographic irregularities. Densely matted algae in very shallow water may be restricted in their production by low carbon availability as well as light limitation. Rapid photosynthetic depletion of inorganic carbon is not balanced by diffusion in or respiratory replacement of more inorganic carbon. Photosynthesis of algae was performed in the 1.5-liter chambers as described in Chapter Two. The 30 $^{o}/_{oo}$ synthetic sea water had been slightly acidified and flushed for a brief period to lower the dissolved inorganic carbon concentration to levels measured in water from mudflat pools at low tide using the method of Strickland and Parsons (1968). The algal densities used in the incubations were higher than for other photosynthetic measurements, but quite low when compared to those encountered in mudflat pools with algal mats. Also, the magnetic stir bars turned at the slowest speed permitted.

Submerged Production

Total submerged production will be some combined function of the duration of daylight submergence, the photosynthetic standing crop, and the photosynthetic rate of algae under estuarine conditions of variable salinity, temperature, and light intensity.

Daylight Submergence

The computer program that provided the estimates of daylight exposure was used to predict daylight submergence at the different tidal elevations studied. Since increasing depth of the water column will reduce light intensity at the mudflat surface, the duration of light-saturated photosynthesis will be somewhat less than the total duration of light submergence for lower intertidal levels. Accordingly, the computer prediction of daylight submergence was diminished by an amount of time with nonsaturating light intensities. This was considered to include periods just after sunrise and just before sunset, as well as periods when light attenuation by the water column restricted photosynthesis. An average extinction coefficient ($k = 0.689$) was computed from multiple Secchi depth measurements (mean: 2.077 ± 0.497 meters, $N = 20$) after Walker (1980).

Photosynthetic Biomass

As rising tides lift the buoyant, gas-containing filamentous algae off of the mudflats, the branches are displayed horizontally in the current. Flowing water will cause fluttering and undulation of the algae, enhancing the even display of chlorophyll by all parts of the mat. Thus, all of the standing crop may be considered to be potentially photosynthetic during periods of submergence in daylight.

Since algal standing crop was only measured at monthly intervals, a daily adjustment of photosynthetic biomass was performed prior to estimation of total production, as suggested by Brinkhuis (1977). A geometrically increasing or decreasing function was used for the

interpolation of algal biomass, for the entire thalli of the Enteromorpha and Ulva species found in the study mats are photosynthetic. The rate of population growth or decline was calculated from the ratio of the standing crop at one month with that of the next, compounded for the number of days between field censuses. In an attempt to eliminate some of the wide variation found between individual field samples, the mean biomass was calculated for each of the four areas censused at each of the five elevations studied. Thus, production estimates for both submerged and exposed conditions were computed using standing crop information from twenty separate areas.

Submerged Photosynthesis

Photosynthesis of submerged algae may depend upon inorganic carbon availability, temperature, salinity, light intensity, and algal density. Measurement of the total dissolved inorganic carbon (Strickland and Parsons, 1968) in flowing water from the field study site suggests that truly submerged algae (as opposed to algae in very shallow pools at low tide) will not be carbon limited. Water temperatures varied only a few degrees around a mean of 16° from June to October; therefore, photosynthetic incubations were held at this temperature in a water bath. Salinity of South Slough water varied between 2 °/oo (March) and 30 °/oo (July through September) for the growing season of 1982. Synthetic sea water was prepared for laboratory measurements of photosynthesis with salinities between 2 and 35 °/oo (American Optical Refractometer).

P-I curves presented by King and Schramm (1976) indicate that Enteromorpha spp. possess compensation light intensities of about 1 mW cm^{-2} and saturation light intensities of about 10 mW cm^{-2}. Photosynthesis by algae in the present study was performed outside over a range of light intensities from 75 mW cm^{-2} (midday sunshine in July and August) to 3 mW cm^{-2} (overcast late afternoon in October). Light was measured with a Kahl Scientific Instrument Corporation photometer, internally corrected for PAR.

Algal density in the 1.5-liter chambers ranged from 0.1 to 0.5 g dry wt $liter^{-1}$. Since algal standing crop was measured in the field surveys with units of g dry wt m^{-2}, a conversion based on field observations was performed to permit extrapolation of laboratory-measured production rates. Algal filaments bend before the tidal currents in all but very slack waters; therefore, the algal mat does not occupy the entire depth of the water column when submerged. Bending of seagrasses approaches a constant angle over a range of current speeds (Fonseca et al., 1982). Similarly, the depth of water, and therefore the volume of water, occupied by an algal mat will vary about some mean value that depends upon the current speed, the standing crop, and the length of the algal filaments. When the field study site was submerged, locations of known standing crop were visited either by wading or snorkeling, and the height above the mudflat surface to which algae floated was measured.

Results and Discussion

Algal Mat Dynamics

An observation from the first year of field sampling is the predictive relationship between algal fresh weight and algal dry weight (Figure 16). While there are slight differences between species, consideration of all commonly encountered green macroalgae from the study site produces a function that may be used in conversion from fresh weight to dry weight, and, with a reciprocal relationship, from dry weight to fresh weight.

There is a distinct seasonal pattern of total algal abundance, with initial growth beginning in May, maximum abundance in August, and a nearly complete decline by late November (Figure 17). The standing crops observed from March through June are quite similar for 1981 and 1982, but from July on, the 1982 algal populations were much larger. The major difference in 1982 was the bloom at the 3.0-foot level of filamentous forms (Figure 18). As each of the two growing seasons proceded, the algal mat was initially confined to the lowest elevations (MLLW and 1.0 feet), but steadily progressed upwards through the summer months, until well over half of the algae occurred at elevations of 3.0 feet or higher. While the mean standing crop in August 1982 was about 310 g dry wt m^{-2}, the mean biomass at 3.0 feet was about 750 g dry wt m^{-2}, and the maximum biomass within one of the four areas at that elevation was nearly 960 g dry wt m^{-2}; the algal mat on the exposed mudflat was more than 10 cm thick in some places. The filamentous species, *E. clathrata* and *E. prolifera*, declined dramatically in September 1982, and the sheet-like

Figure 16. Algal dry weight as a function of fresh weight: the species considered are those encountered in typical green algal mats; i.e., Enteromorpha prolifera (open circles), E. clathrata (filled circles), E. linza (triangles), and Ulva (diamonds).

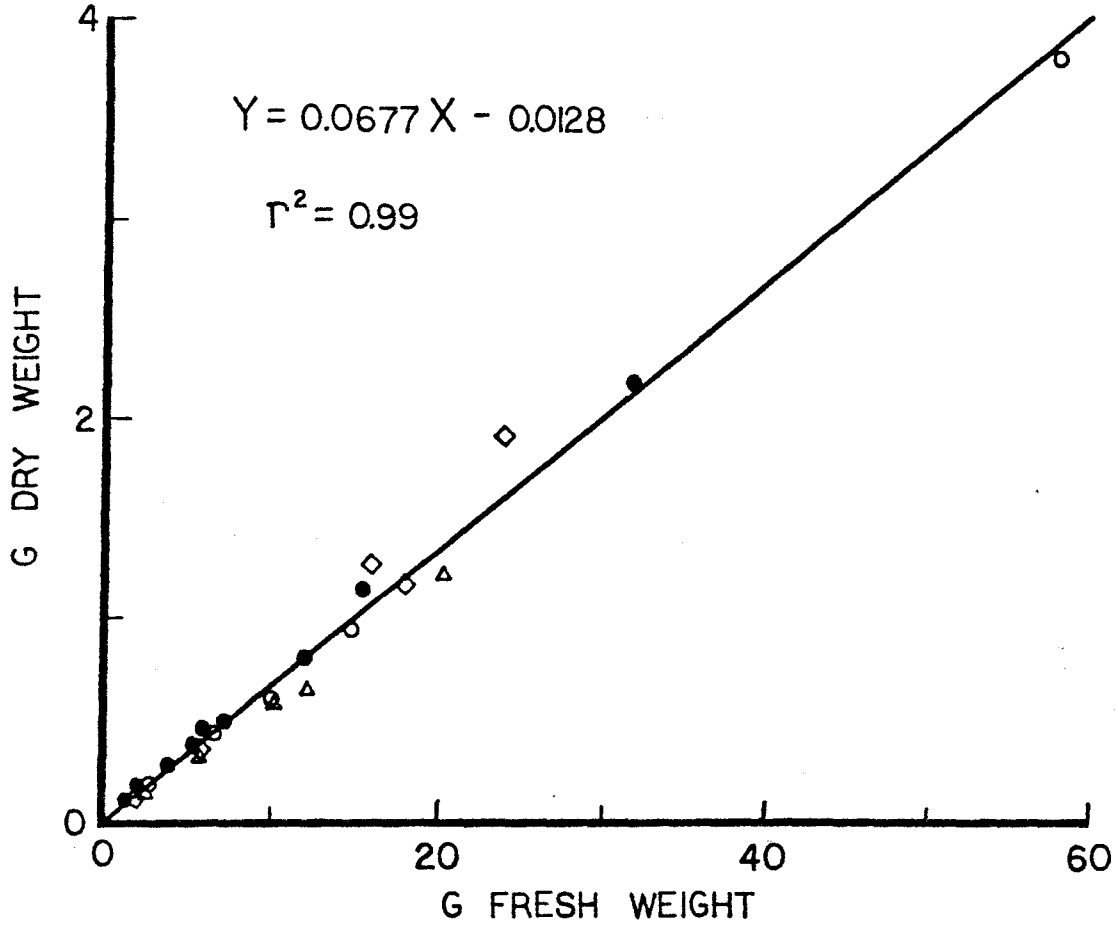

Y = 0.0677 X - 0.0128
r² = 0.99
G DRY WEIGHT
G FRESH WEIGHT
0
2
4
0
20
40
60

Figure 17. Annual pattern of algal mat standing crop at the study site in South Slough: the uppermost line indicates the mean value for the entire site, and the inner delineations represent the relative contributions from the five tidal elevations sampled.

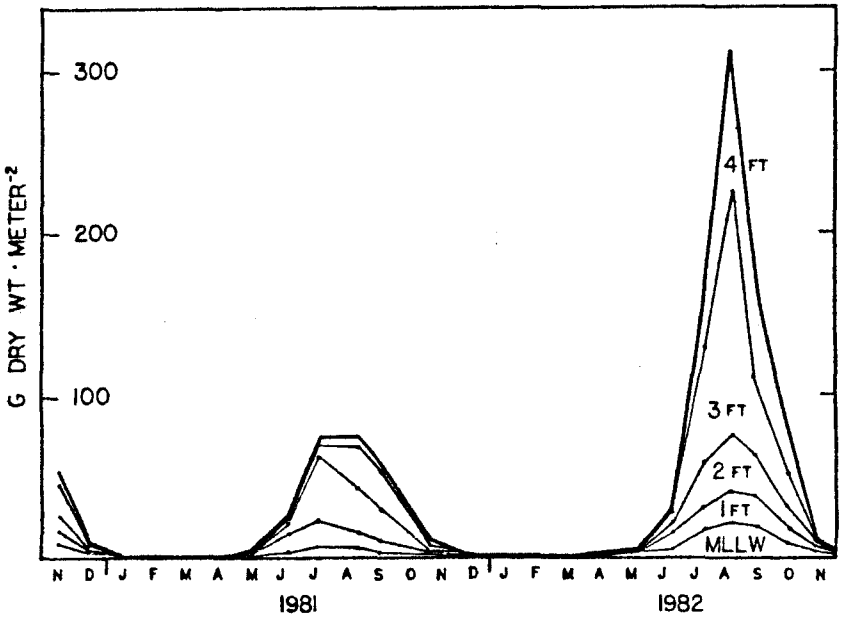
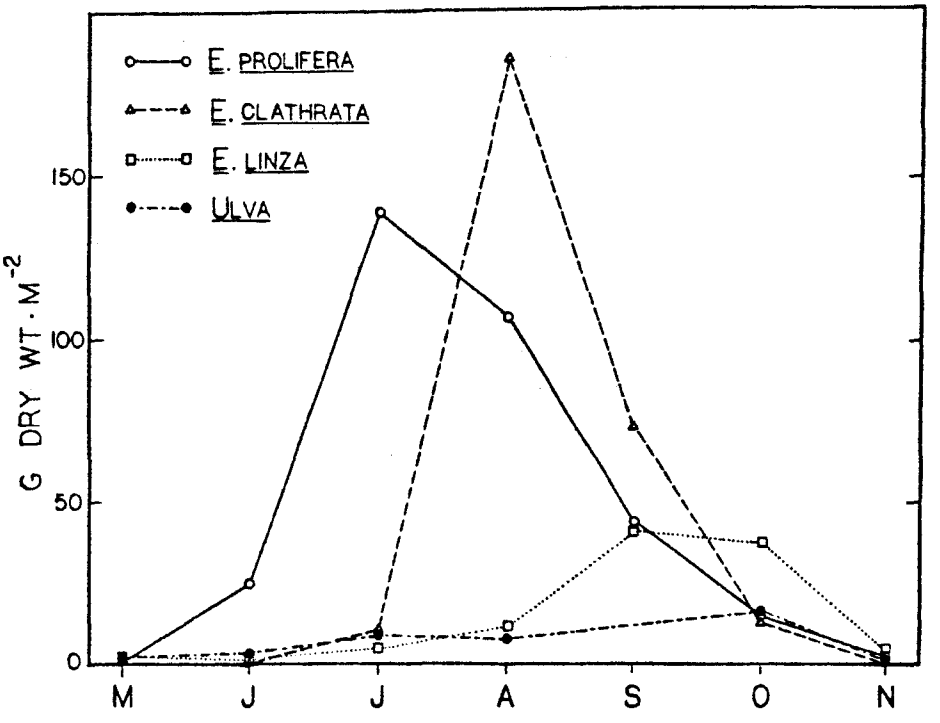

300
200
100
G DRY WT · METER⁻²
4 FT
3 FT
2 FT
1 FT
MLLW
N D J F M A M J J A S O N D J F M A M J J A S O N
1981
1982

Figure 18. Changes in species composition in the algal mat sampled in South Slough during the growing season of 1982.


E. PROLIFERA
E. CLATHRATA
E. LINZA
ULVA
G DRY WT · M⁻²
150
100
50
0
M
J
J
A
S
O
N

forms, E. linza and Ulva spp., increased in abundance (Figure 18). E. linza was the most common species through the fall.

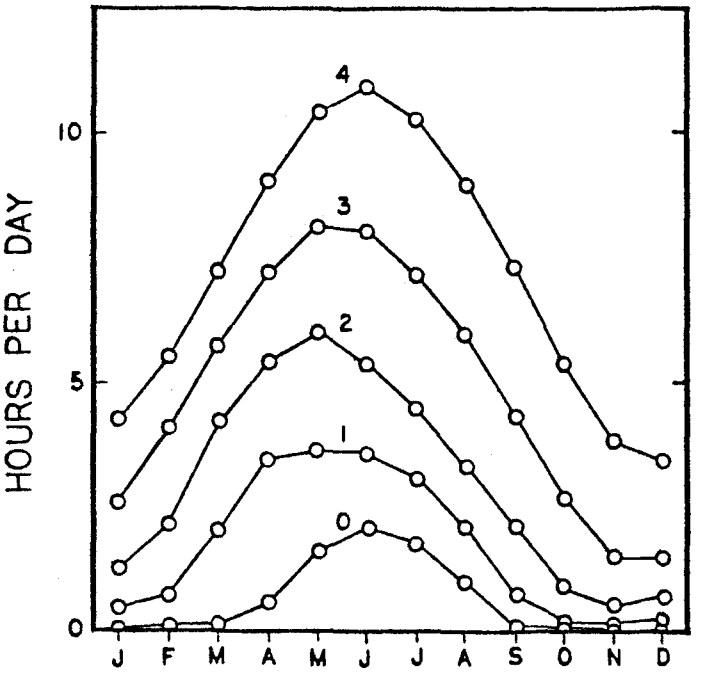
The autumn decline was caused primarily by gales that stripped algae off of the mudflats. Fitzgerald (1978) found that increased surf intensity caused repeated declines in E. clathrata populations on Guam. Also, algae became increasingly buried by sediments in Coos Bay, both from algae-enhanced deposition (Frostick and McCave, 1979) and from wave-induced sediment transport. The algal mat may have become more susceptible to physical disruption owing to declining vigor. Favorable light intensities in summer months permit the growth of a thick algal mat; rapid decreases in light intensity in the fall (shorter daylength and increased cloud cover) may subject the lower sections of the algal thalli to below-compensation light conditions. Degradation of the attaching portions of the algae permits the upper, still-buoyant layers of algae to become detached (Kier and Todd, 1967). Masses of green algae are observed floating about the estuary as macrophytoplankton. These algae are still photosynthetic and reproductive; the primary production by this algal component is unknown, but it must be substantial.

Estimated Algal Production

Exposed Production

The time available for photosynthesis by emersed algae on the Coos Bay tideflats is indicated in Figure 19. Note that in May and June, when the algal populations are just beginning to expand, daylight exposure is at its maximum for the year. As the growing season progresses, fewer hours of daylight emersion confront the algae. The use of predicted

Figure 19. Mean daylight exposure (month average) for the five levels at which algal standing crop was monitored: tide information is from the Humboldt Bay reference station, corrected to the Coos Bay entrance.


HOURS PER DAY
10
5
0
4
3
2
1
0
J F M A M J J A S O N D

tide curves should be valid in this situation, for the protected mudflat receives only small waves, and the slope of the study area is quite gentle (2 meters vertical drop in 150 meters). Otherwise, the computer estimates of exposure and submergence might be substantially different from actual conditions (Druehl and Green, 1982).

Desiccation of exposed algae is highly dependent upon standing crop and the duration of exposure (Figure 20). If the compressed mat of algae on the mudflat is less than about one cm thick, internal water is rapidly lost, and photosynthesis declines (Figure 3). If the algal mat is more than one or two cm thick, loss of water is not as severe, and photosynthesis may continue if sufficient light is available. While slight desiccation is known to enhance photosynthesis by midlittoral fucoid algae (Johnson et al., 1974; Quadir et al., 1979), water loss inhibits photosynthesis in thin sheet-like or filamentous algae (Imada et al., 1970; Wiltens et al., 1978; Quadir et al., 1979). Enteromorpha spp. are in this latter group of algae.

The emersed algal mat will attenuate light such that saturation and compensation light intensities are found within the upper few cm of the mat itself (Figure 21). Thus, only the surface layers (which are those most susceptible to stresses of desiccation and rainfall) may be photosynthesizing at low tide. Photosynthesis by the estuarine green alga Cladophora also declines within thick mats (Bach and Josselyn, 1978; Cordon et al., 1980).

If algae come to rest in shallow puddles on the mudflat surface, they will not be subject to desiccation, but light attenuation will be just as great. Observations of low inorganic carbon concentrations in

Figure 20. Enteromorpha spp.: degree of desiccation of algae as a function of standing crop, after 1 hour of desiccation (dashed line) and 3 hours of desiccation (continuous line). The plotted points and regression equation are for t = 3 hours of desiccation.

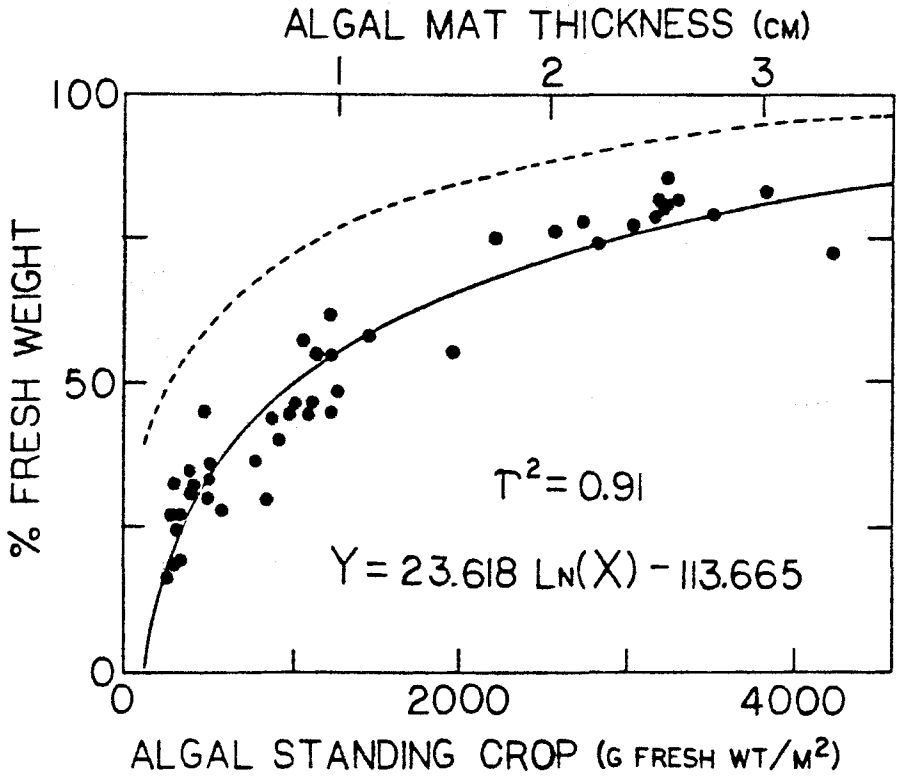
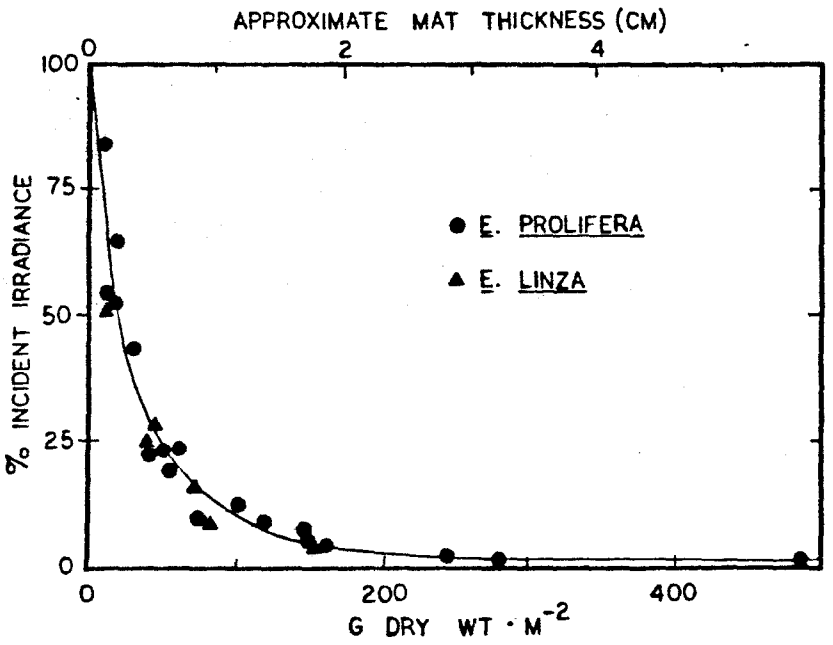

ALGAL MAT THICKNESS (cm)
1
2
3
100
50
0
% FRESH WEIGHT
$r^2 = 0.91$
Y = 23.618 Ln(X) - 113.665
0
2000
4000
ALGAL STANDING CROP (g fresh wt/m²)

Figure 21. Enteromorpha spp.: light attenuation (percent of surface irradiance) by an algal mat.


APPROXIMATE MAT THICKNESS (CM)
0
2
4
100
75
50
25
0
% INCIDENT IRRADIANCE
E. PROLIFERA
E. LINZA
0
200
400
G DRY WT · M^{-2}

pools with large amounts of algae suggest that photosynthesis will be carbon limited. Indeed, an inverse relationship between algal density and photosynthetic rate does occur with low carbon availability (Figure 22). During the three hours of the experimental incubation, well over 90% of ^{14}C-carbonate was fixed by the algae.

Thus, several factors combine to reduce the magnitude of exposed algal production on the Coos Bay tideflats. First, the time available for such production declines through the growing season at all elevations. Secondly, desiccation of algae at low standing crop reduces photosynthesis. Thirdly, light attenuation within algal mats of greater standing crop restricts photosynthesis to the upper layers of the mat. Finally, severe carbon limitation of algae in shallow pools of water restricts photosynthesis. In contrast, the population of Enteromorpha clathrata studied by Shellum and Josselyn (1982) in San Francisco Bay showed its greatest production when tidal exposure (frequency of daytime low tides) was at a maximum.

Submerged Production

The time available for submerged photosynthesis gradually increases through the entire growing season for all levels at which algal production was estimated (Figure 23). This is due to the interaction between the tidal cycles and seasonal daylength changes as discussed in the Appendix. From July on, only the uppermost tidal elevation sampled (4.0 feet) experiences a greater amount of daylight exposure than daylight submergence; all lower levels spend considerably more time submerged when light intensity permits net photosynthesis.

Figure 22. Enteromorpha prolifera: photosynthesis by carbon-limited algae as a function of algal density.

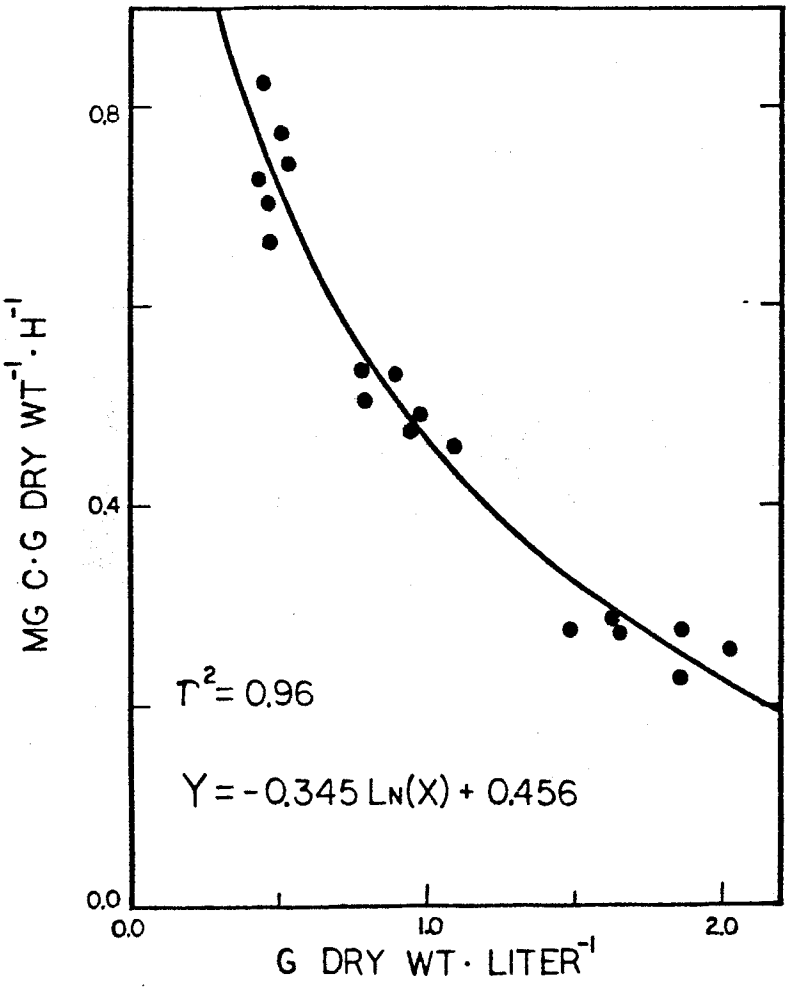
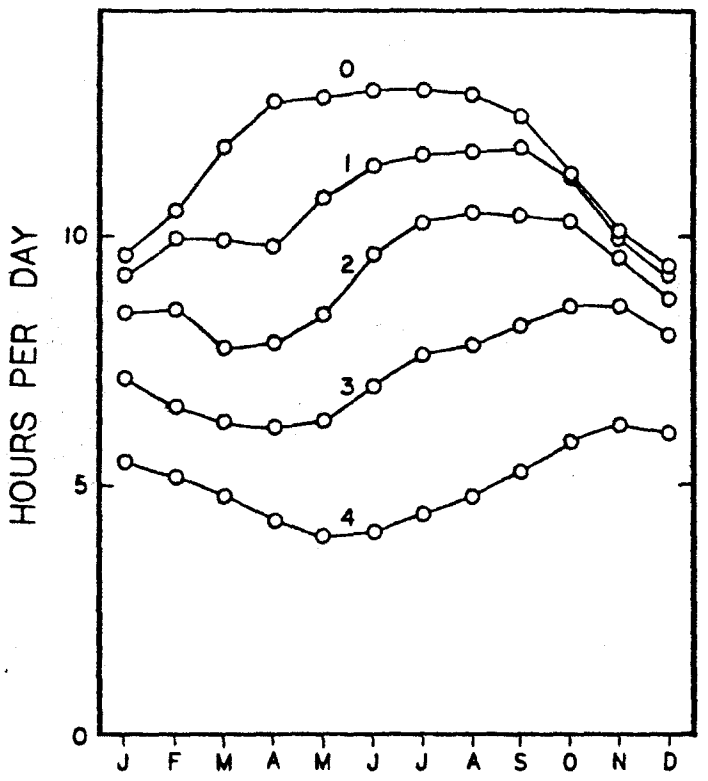

0.8
0.4
0.0
0.0
1.0
2.0
MG C·G DRY WT^{-1}·H^{-1}
G DRY WT · LITER^{-1}
r^2= 0.96
Y = -0.345 Ln(X) + 0.456

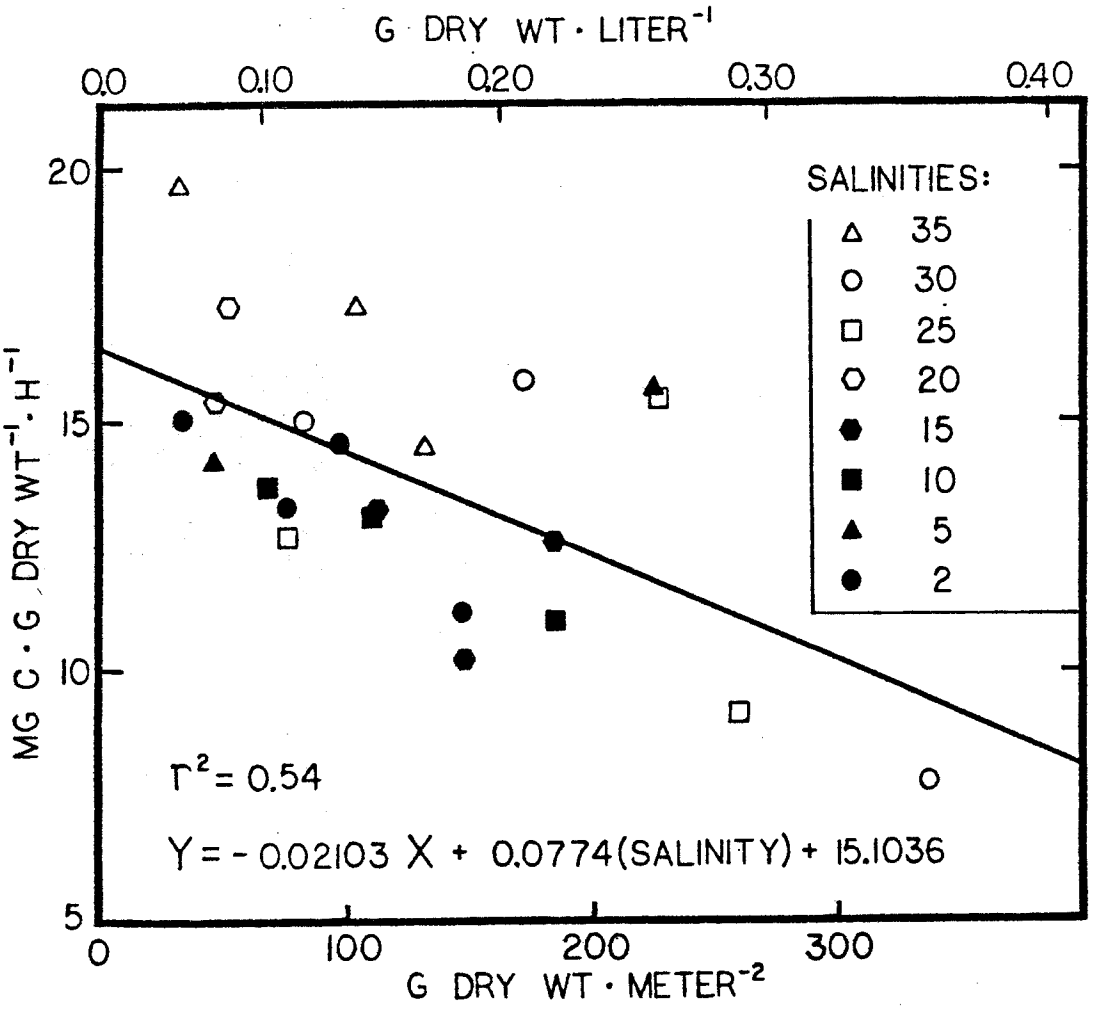
Figure 23. Mean daylight submergence (month average) for the five levels at which algal standing crop was monitored: tide information is from the Humboldt Bay reference station, corrected to the Coos Bay entrance.


HOURS PER DAY
0
5
10
J F M A M J J A S O N D
0
1
2
3
4

Photosynthesis by green algae from the South Slough study site is highly dependent upon algal density and salinity (Figure 24). The decline in carbon fixation at higher density is probably due to combined effects of self shading, competition for inorganic carbon and other nutrients, and less boundary-layer disruption (Westlake, 1967; Littler, 1979; Gordon et al., 1980). Net carbon fixation is slightly lowered under less saline conditions; this may be due to increased loss of dissolved organic carbon (see Chapter Two). Others have also found that salinity has a significant but minor effect on photosynthesis in Enteromorpha spp. (Fitzgerald, 1978; Reed and Russell, 1979; Shellum and Josselyn, 1982).

Photosynthetic incubations in the present study were performed over a range of light intensities from 3 to 75 mW cm^{-2}. When effects of algal density and salinity are factored out, it appears that photosynthesis is light limited below aobut 8 mW cm^{-2}, saturated between about 10 and 45 mW cm^{-2}, and photoinhibited above 60 mW cm^{-2}. These values generally agree with the information in P-I curves presented by other researchers for green macroalgae (e.g., King and Schramm, 1976; Fitzgerald, 1978; Arnold and Murray, 1980; Shellum and Josselyn, 1982) if the different units of light intensity are approximately interconverted (Arnold and Murray, 1980). Thus, submerged algae from the study site can maintain rapid carbon fixation over a wide range of light intensities and salinities observed during the growing season. Production per unit biomass is diminished at high standing crop levels, so there may be some upper limit to biomass for a given set of environmental conditions.

Figure 24. Enteromorpha spp.: submerged photosynthesis as a function of algal density (converted from g dry wt liter^{-1} to g dry wt m^{-2}) and salinity (^{o}/oo).


G DRY WT · LITER⁻¹
0.0
0.10
0.20
0.30
0.40
20
15
10
5
MG C · G DRY WT⁻¹ · H⁻¹
SALINITIES:
35
30
25
20
15
10
5
2
r² = 0.54
Y = - 0.02103 X + 0.0774(SALINITY) + 15.1036
0
100
200
300
G DRY WT · METER⁻²

Estimated Algal Mat Production

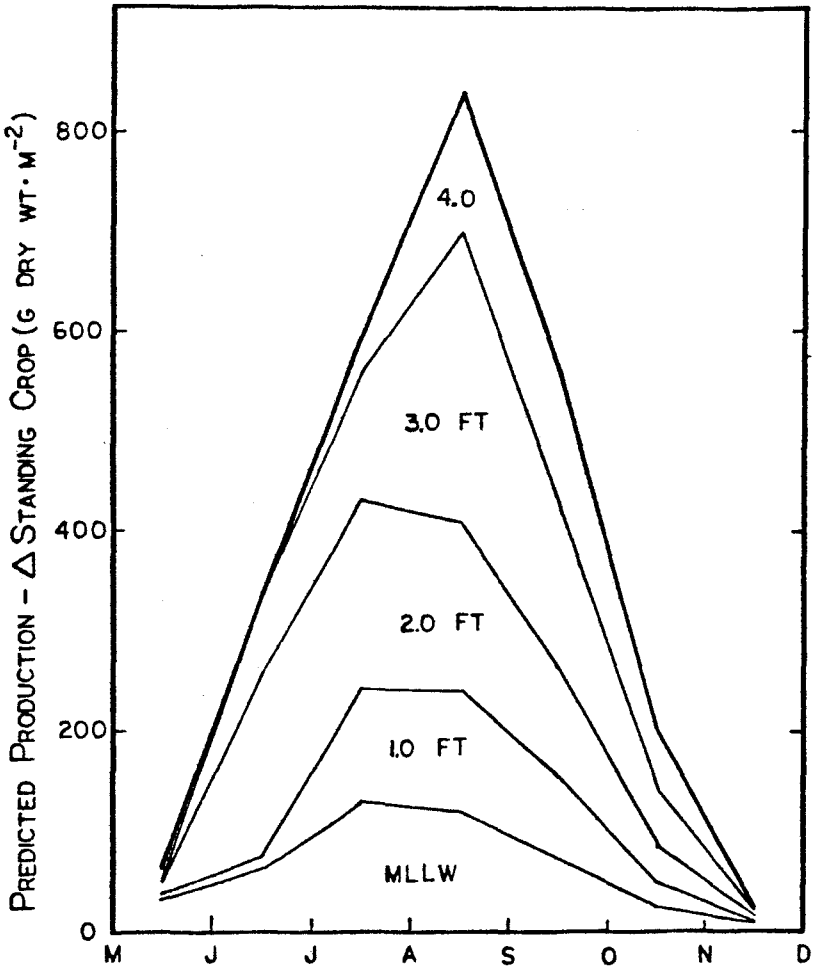
Using the functions derived above, exposed and submerged production were computed on a daily basis. Expected standing crop was corrected between monthly field censuses in each of the four areas at the five elevations. Table 3 indicates the predicted algal mat production for each level and time interval considered. Two points are of interest. First, predicted production is very high, with an average over all elevations of about 2650 g dry wt m^{-2} for the year. This estimate places these algal mats among the most productive plant systmes for which total production estimates have been made (cf. Mann, 1972). Secondly, the estimated contribution of exposed production is quite low, even at the highest elevation considered. This suggests that conditions of exposure inhibit algal mat growth in the Coos Bay estuary, and yet it is the uppermost elevationsthat achieve the greatest standing crop and the highest production estimates. Shellum and Josselyn (1982) observed maximum production of a green algal population with maximal daylight exposure, and suggested that the great turbidity of San Francisco Bay waters inhibit submerged production through light limitation.

The predicted production in the present study is much higher than observed maximum standing crop. The ratio of predicted production to change in standing crop for the first months of the growing season indicate turnover rates of 1.5 to 8.7. The difference between predicted growth and the observed change in standing crop should indicate excess production that is available to the estuarine system during the growing season. This difference is illustrated in Figure 25; note that excess

Table 3. Estimated algal mat production for the 1982 growing season, given for each elevation studied and for each month interval between field censuses. The first value is the total estimated production (g dry wt m^{-2}), the second value in parentheses, is one standard deviation (calculated from the four areas per level), and the third value is the percent of total production which occurs while exposed.

	4.0 ft	3.0 ft	2.0 ft	1.0 ft	0.0 ft
May					
	3.46	68.81	106.02	95.63	148.69
	(0.87)	(58.54)	(102.64)	(58.65)	(18.23)
	0.58	3.12	1.95	1.23	0.59
June					
	101.82	745.54	522.09	522.98	426.18
	(97.42)	(266.69)	(349.99)	(111.70)	(268.71)
	10.21	6.87	2.96	2.03	0.96
July					
	536.92	1037.54	965.75	587.13	675.55
	(203.88)	(142.98)	(385.11)	(42.53)	(258.49)
	15.68	7.07	2.54	1.33	1.29
August					
	600.92	914.87	818.18	576.04	595.98
	(187.52)	(301.94)	(223.54)	(148.73)	(160.69)
	14.72	6.89	2.02	1.33	1.29
September					
	527.99	650.90	493.98	388.92	342.98
	(163.00)	(301.94)	(193.96)	(96.77)	(205.94)
	8.73	3.62	1.28	1.42	1.38
October					
	171.95	171.34	117.69	78.34	95.09
	(75.47)	(71.21)	(36.20)	(24.01)	(45.86)
	5.60	2.52	1.57	1.51	1.75
November					
	8.84	13.18	11.89	8.09	26.69
	(3.49)	(3.91)	(4.88)	(4.19)	(9.20)
	1.70	0.91	1.84	0.62	1.31
December					
Total	2040.05	3602.18	3035.60	2257.14	2311.15
% Exposed	11.71	6.05	2.20	1.67	1.18

Figure 25. Estimated algal mat production less observed change in standing crop for monthly intervals in the 1982 growing season. The upper line is the mean for the entire field study site, and the internal delineation indicates the relative contribution of the five tidal elevations monitored.

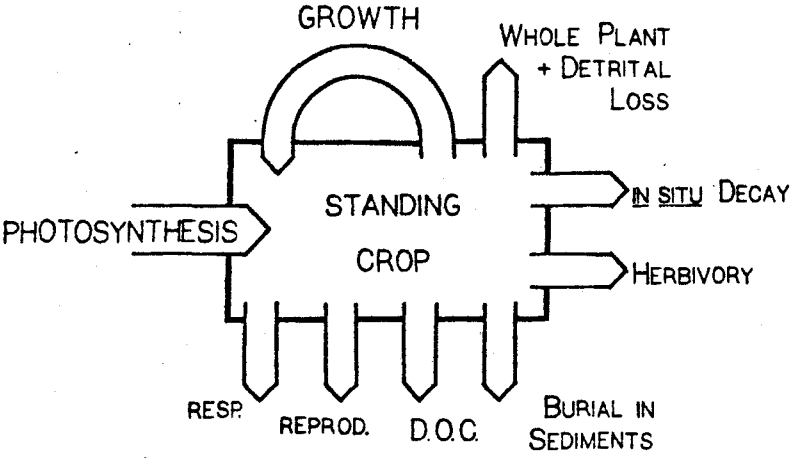

PREDICTED PRODUCTION - Δ STANDING CROP (G DRY WT · M⁻²)
800
600
400
200
0
4.0
3.0 FT
2.0 FT
1.0 FT
MLLW
M
J
J
A
S
O
N
D

production increases until the biomass maximum between August and September, and declines just as rapidly. Separation of the excess production by elevation indicates that the middle intertidal zone is most important for total algal contribution to the estuary. Also, much of the excess production after August consists of algae that had been present on the mudflats but are being stripped away by wave action. Thus, these green algal mats partially function as generalized estuarine macrophytes, which store organic matter during the summer and contribute it to the estuary after the growing season.

Potential Fate of Algal Production

Total algal production can enter the estuarine system in various ways (Figure 26). An initial loss of gross production is the respiratory consumption of photosynthate by the algae themselves. Liberation of reproductive cells (gametes and swarmers) involves the specialization and release of formerly photosynthetic tissues. Fixed carbon may be lost by the algae in the form of dissolved organic carbon (DOC). Algae may become buried in sediments, only to be returned to the estuary some time later through wave-caused sediment turnover. Excessively shaded or epiphytized algae may become senescent while still within the mudflat population; their decay involves the loss of DOC and particulate organic matter that enters the detrial pool. Old cell wall materials may be sloughed off, and grazers may fragment algae while feeding. Storm waves and rapid tidal currents can physically remove entire algal thalli and groups of thalli. These algae may drift along the bottom of the bay as they decompose or they may continue to float about the estuary in large

Figure 26. Schematic representation of potential outputs of total algal production: the rates of the various outputs may change with time and location.


GROWTH
WHOLE PLANT
+ DETRITAL
LOSS
PHOTOSYNTHESIS
STANDING
CROP
IN SITU DECAY
HERBIVORY
RESP.
REPROD.
D.O.C.
BURIAL IN
SEDIMENTS

masses, still photosynthesizing and reproducing. The absolute and relative magnitudes of these various fates will change through time and in space over the mudflat.

I have made a number of assumptions in estimating the amount of algal production that has entered the estuary in the ways described above. First, I used a respiration value of 10% of total production (Kanwisher, 1966; King and Schramm, 1976). Secondly, since I have been unable to find quantitative results on propagule release, I have visually estimated that 5% of total algal production entered the estuary in this way; algae held in the laboratory will often release swarmers. These cells may be consumed by various particle feeders, they may decompose, or they may settle out to be eaten by benthic grazers or to survive and grow in the population.

Since the quantity and quality of DOC release depends upon varying conditions in the estuary, I assumed that a larger proportion of production was lost as DOC at the higher elevation (more frequent tidal resubmergence, rainfall, and reduced salinity). Most of this algal production will quickly be consumed by heterotrophic microbes in the water column and on the mudflat surface. Thus, a large portion of algal production is rapidly cycled through the estuary, with very short residence times inside the algal cells, in the pool of DOC, and perhaps in the microbial cells as well.

Burial of algae by sediments will partially be a function of standing crop (increased current baffling), the frequency of slack tides, and resuspension by tidal currents. Field observations indicate that the 2.0- and 3.0-foot levels experienced considerable burial of algae.

Price and Hylleberg (1982) found between 8 and 20% of the standing crop of an Ulva population buried in sediments after the fall decline.

It is likely that small grazers consume more algal biomass than large herbivores in the estuary, with the exception of locally important grazing by geese. Price and Hyllegerg (1982) estimated that grazing by amphipods might consume 112 g dry wt m^{-2} $month^{-1}$ of Ulva in False Bay, Washington. Since these grazers also fragment algal filaments while feeding, they have an effect upon standing crop that is greater than that predicted solely by their consumption (Warwick et al., 1982). To quantify these two effects of grazing, I held algae in replicate finger bowls under three conditions: controls with no grazers, algae with amphipods (Gammaridae), and algae with snails from the field site (Assiminea californica). After one week, I measured the difference in algae weight, the weight of the grazers, and the fraction of algal filaments that had not been consumed but that had been fragmented. These results are presented in Table 4; note that these common (and sometimes extremely abundant) grazers produce a greater weight of algal fragments than of algal tissues consumed. It is likely that these animals continue to feed on algae even at low tide on overcast or cool days, while on warm or sunny days they are observed huddled against the mudflat surface underneath all algae. Nearby Zostera beds may harbor additional grazers that can feed on algae at the lower elevations. The green-lined shore crab, Pachygrapsus crassipes, has been observed feeding on algal strands at high tide.

Decay in situ of algae occurs most often at the middle elevations, where extremely high standing crop causes self shading. It is possible

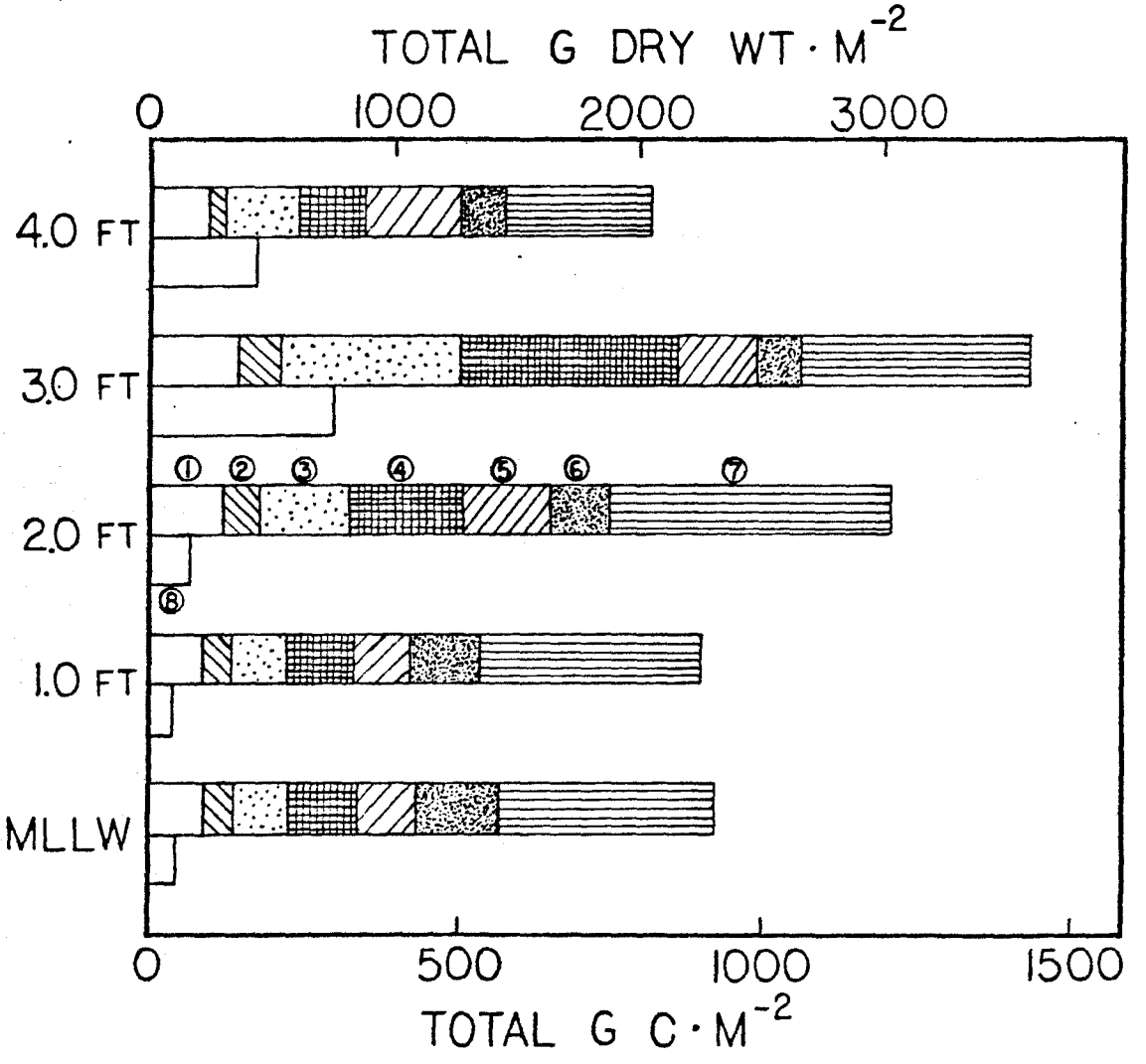
Table 4. Enteromorpha spp.: effects of small herbivore grazing. Treatments held for one week at 14° C and 10L:14D. Means and standard deviations from five replicates in each treatment.

	Control	Snails	Amphipods
Starting algal dry weight (mg)	196.78 ± 40.61	191.70 ± 12.72	154.35 ± 29.07
Ending algal dry weight (mg)	192.94 ± 32.37	152.70 ± 20.17	94.24 ± 28.11
Control-corrected weight change (%)	---	- 18.39	- 36.99
Grazing rate: mg algae mg grazer^{-1} week^{-1}	---	1053.08 ± 553.72	1902.17 ± 700.71
% remaining algae in fragments	---	33.27 ± 21.73	61.06 ± 34.25

to compare an algal mat with a population of phytoplankton in the water column; for a given light intensity, there will be some compensation depth (mat thickness) beneath which that algal cells are net respirers, and some critical depth or thickness that, if exceeded by an algal population, will limit production by population dieback. The green macroalgae being studied can quickly respond to favorable light intensities by growing. If daily light intensities are suddenly lowered by heavy cloud cover, the algal mat may automatically exceed its critical thickness, and the lower portions (which probably had been beneath the "compensation thickness") will decay. With loss of the attaching section of algal, large masses will readily be borne away on tidal currents (Kier and Todd, 1967) or if wave chop or gale-force winds cross the mudflat with the rising and falling tides. Thus, in situ decay and whole-plant loss may well be interdependent.

Based on these considerations, I propose that the total predicted algal mat production is used up or enters the estuarine system in the relative proportions indicated in Figure 27. The three most important outputs are release of DOC, burial in sediments, and whole plant loss. It has been stated that DOC is a form of algal production that is immediately used within the estuary. Buried materials may decompose completely, or it may serve as a store of organic matter that is partially consumed in the benthos and partially in the particulate-based food web after it is made available by wave action. Since floating algal mats continue producing, the importance of green algae should actually be larger than estimated here. These macrophytoplankton will eventually sink and decay, breaking up as they drift along the bottom as marine tumbleweed, or they will wash up on a shoreline, to

Figure 27. Total production of green algal mats as a function of elevation, and the estimated outputs of total production in various forms: (1) algal respiration (2) reproductive release of swarmers (3) loss of DOC during photosynthesis (4) burial of algae in sediments (5) herbivory (6) in situ decomposition (7) whole plant and fragment loss (8) maximum observed standing crop.


TOTAL G DRY WT · M^{-2}
0
1000
2000
3000
4.0 FT
3.0 FT
2.0 FT
1.0 FT
MLLW
①
②
③
④
⑤
⑥
⑦
⑧
0
500
1000
1500
TOTAL G C · M^{-2}

be consumed by intertidal detritovores.

In summary, the green algal mats of the Coos Bay estuary are extremely productive, as productive as the oft-cited salt marshes and seagrasses. They lose much of their initially fixed carbon as DOC; estuarine fluctuations increase the magnitude of release. The substances lost by the algae are quickly utilized by estuarine microbes, whose activity is essential to high estuarine production. Since production of algal mats becomes available to the estuary in a wide variety of forms, they serve to moderate the actual peak of production observed; some algal outputs are used instantaneously, others are consumed during the growing season, while others remain available for consumption by various guilds of animals long after the algae are not on the tideflats.

APPENDIX

Annual Patterns of Diurnal Exposure and Submergence in Regions with Mixed Semidiurnal Tides

One of the most obvious characteristics of an intertidal environment is the occurrence of organisms in distinct vertical bands or zones. Vertical zonation has long been attributed to the amount of exposure and submergence that different levels of the shoreline experience with the cyclic rising and falling of the tides. Arguments have been made for and against the role that the tides play in restricting organisms to certain vertical ranges (e.g., Doty, 1946; Chapman, 1973; Underwood, 1978; Druehl and Green, 1982; Swinbanks, 1982). It is now generally acknowledged that an organism's distribution is partially due to its own tolerances to stressful conditions and to those of its chief competitors, predators, and prey. Differences in vertical position have been found to affect an organism's growth and survivorship owing to food availability (Frank, 1965; Sutherland, 1970), competition (Connell, 1961), and predation (Paine, 1974).

Organisms may exhibit different behavioral or physiological responses in light versus dark for conditions of exposure or submergence. Algae photosynthesize at different rates in air versus water (e.g., Johnson et al., 1974; Quadir et al., 1979; Holmes and Mahall, 1982). Limpets may or may not graze depending upon the potential for

desiccation if exposed in daylight or upon the risk of dislodgement while submerged (Hawkins and Hartnoll, 1982). Foraging activity of intertidal crabs may decrease in day versus night under conditions of tidal exposure (Batie, 1983). The time available to shorebirds for feeding in the intertidal zone may be limited by impending darkness and/or submergence (e.g., Recher, 1966; Burger et al., 1977; Gerstenberg, 1979; Frank, 1982; Burger, 1983). Coral reef communities experience severe stress if maximum tidal exposure coincides with high insolation or with daily patterns of rainfall (Pugh and Raynor, 1981). Thus, the condition of daylight exposure is often invoked as being important to the distribution or activity of an intertidal organism. However, most quantitative analyses of tidal emersion consider the average exposure and submergence that occurs over an entire year (Underwood, 1978; Hartnoll and Hawkins, 1982), the maximum duration of submergence or exposure over one of many years (Doty, 1946; Swinbanks, 1982), or make no distinction between light and dark when calculations are made over time scales of days to weeks (Sutherland, 1970; Druehl and Green, 1982; Swinbanks, 1982).

In the course of the present research on the productivity of intertidal macroalgae, I found it was necessary to differentiate between conditions of emergence and submergence during daylight hours. Tide-simulation models were constructed to calculate the durations of exposure and submergence in daylight and darkness for various levels along a gradient of tidal height. In this Appendix, I show the results of these tide simulations and the general applicability of this information in regions that experience mixed semidiurnal tides.

Methods

Computer programs have been assembled in FORTRAN IV and PASCAL to calculate and display information pertaining to the tide curves. All programs are initially based upon a simple tide prediction that fits a sine functions between each successive pair of tides (low-to-high or high-to-low). The water level at each hour is determined and stored in an array. Each program then uses this array, in conjunction with information on the times of sunrise and sunset, in a different fashion. One program computes the durations of exposure and submergence in daylight (sunrise to sunset) and darkness (sunset to sunrise) at 0.1 ft (0.03 m) intervals between -1.0 and +7.0 ft (-0.3 to +2.1 m) relative to Mean Lower Low Water (MLLW), which is the local tide datum. Total durations were computed for each calendar day, as were mean values for each month of the year. Another program calculates the average water level in daylight versus darkness for each day and month. A third program creates a "topographic map" through time of the tide levels.

The raw data for these programs consist of the predicted times and elevations of the tides and the times of sunrise and sunset. This information was drawn from the National Ocean Survey's Tide Tables 1983, High and Low Water Predictions for the Pacific coast of the Americas. While there are distinct shortcomings in the use of tide predictions instead of actual tide records (Druehl and Green, 1982), analyses performed with such information can be valuable, particularly if tide measurements from field study sites are difficult to obtain.

The tide simulations were run for three reference stations from the Pacific coast of the United States that occur at intervals of roughly 8^{o} of latitude (Table 5). Corrections of tidal time and amplitude would be necessary for locations away from reference stations, such as along a coastline or up an estuary. All three reference stations considered here experience mixed semidiurnal tides, in which the two high tides each day attain different elevations, and the two low tides each day recede to different levels (Figure 28A).

Results and Discussion

The portion of an intertidal environment that is submerged changes over the course of hours and days (Figure 28). One may find the overall degree of exposure and submergence expressed in an "emersion curve" (*nderwood, 1978; Druehl and Green, 1982; Hartnoll and Hawkins, 1982). Such curves have been constructed for the San Diego reference station for total 24-hour emersion and for emersion in daylight alone (Figure 29). While there is some monthly variation about the annual mean for the total degree of exposure (Figure 29A), there is greater variation about the an-ual mean of daylight exposure (Figure 29B).

Figure 30 emphasizes these month-to-month changes in day-night submergence and emergence by indicating the patterns at only selected elevations. The influence of seasonal daylength changes is suggested in the pattern of daylight exposure (Figure 30A), in which most tide levels exhibit maxima during late spring or early summer. Correspondingly, most levels experience their longest duration of night exposure

Table 5. Location of tide-data reference stations.

Station	Location	Tide Range (m)		Mean
		Mean	Spring	Tide Level
San Diego, California	32° 43' N, 117° 10' W	1.25	1.74	0.91
Humboldt Bay, California	40° 45' N, 124° 14' W	1.37	1.95	1.04
Port Townsend, Washington	48° 08' N, 122° 46' W	1.58	2.56	1.55

Figure 28. Mixed semidiurnal tides: (A) hourly tide heights showing the pattern of higher high, lower low, lower high, and higher low (B) tide series over 19 days indicating how the higher and lower tides interchange during the period of neap tides.

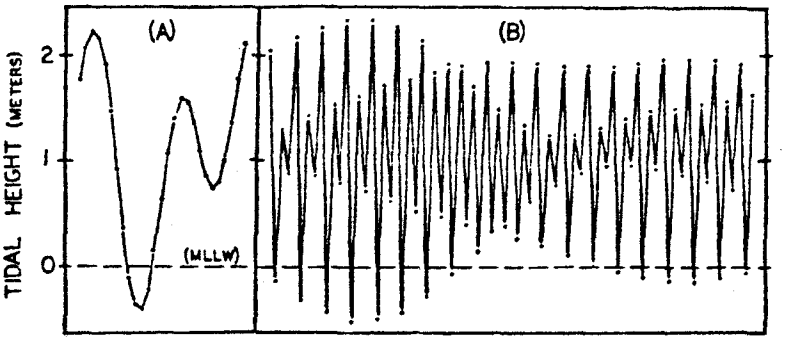

(A)
(B)
TIDAL HEIGHT (METERS)
2
1
0
(MLLW)

Figure 29. Emersion curves for San Diego: (A) diel (B) daylight only- The central, continuous curve in each is the annual mean, while the dashed lines indicate the maximum and minimum range of mean monthly emersion for the year.

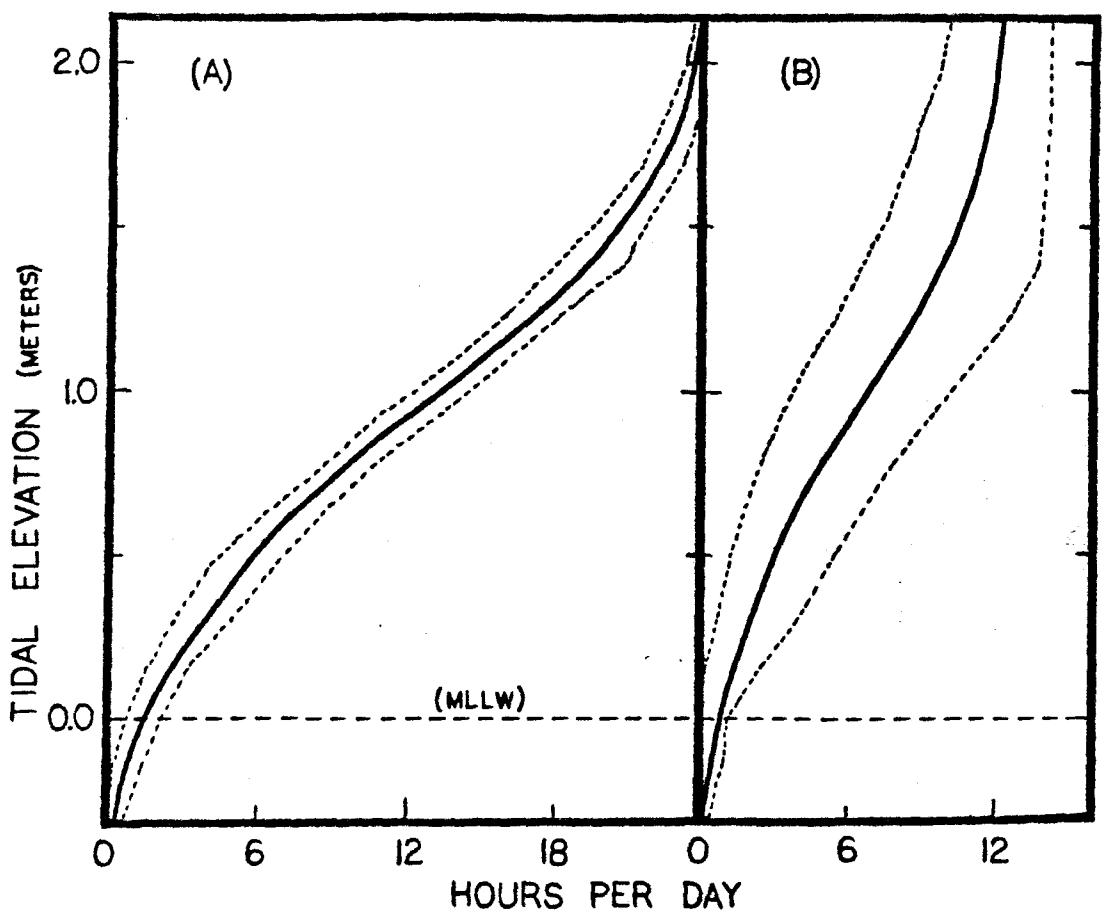
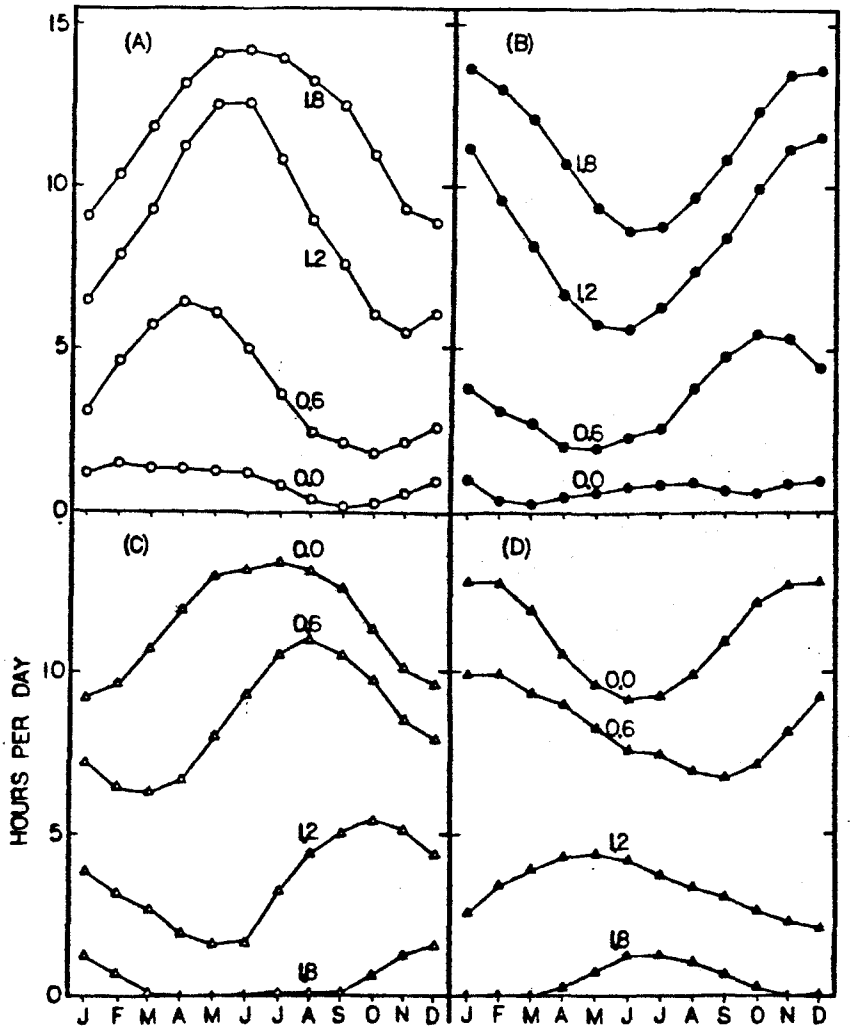

(A)
(B)
2.0
1.0
0.0
(MLLW)
TIDAL ELEVATION (METERS)
0
6
12
18
0
6
12
HOURS PER DAY

Figure 30. San Diego: daily durations (month average) of emergence (circles) and submergence (triangles) in daylight (open symbols) and darkness (filled symbols) at 0.6 m intervals between MLLW and 1.8 m: (A) daylight exposure (B) night exposure (C) daylight submergence (D) night submergence.


(A)
(B)
(C)
(D)
1.8
1.2
0.6
0.0
HOURS PER DAY
15
10
5
0
J F M A M J J A S O N D

in late fall and midwinter (Figure 30B). The patterns of submergence are more complex, with very low tide levels following annual daylength cycles, high tide levels showing an inverse pattern, and intermediate levels phase-shifted between the two extremes (Figures 30C and D).

Figure 31 indicates the comparable patterns for the Humboldt Bay reference station. Again, daylength changes exert greater influence upon the exposure patterns, with light maxima coming in May or June, and dark maxima occurring in November and December. The patterns are slightly more accentuated at Humboldt Bay than in San Diego (cf. Figures 30 and 31); this is due to the increasing tide range and daylength changes at high latitude. The submergence patterns show the same complex configurations observed for San Diego, but they, too, are slightly more accentuated.

Why do the higher tidal elevations receive less daylight submergence in summer, when there is more daylight, than in winter? Along the Pacific coast of North America, the lower low tides are preceded by the higher highs and followed by the lower highs (Figure 28A). Twice each month during the spring tide series this pattern is amplified (Figure 28B). However, the time of day at which the extremely low tides occur also has a distinct annual pattern at a given location on this coastline (Figure 32). At Humboldt Bay, these low tides occur during daylight hours from March through August; the extremely high tides which precede them will thus come mostly during darkness. From September through February the opposite is true, and daylight hours receive both the higher high tides and the higher low tides. There is

Figure 31. Humboldt Bay: daily durations (month average) of emergence (circles) and submergence (triangles) in daylight (open symbols) and darkness (filled symbols) at 0.6 m intervals between MLLW and 1.8 m: (A) daylight exposure (B) night exposure (C) daylight submergence (D) night submergence.

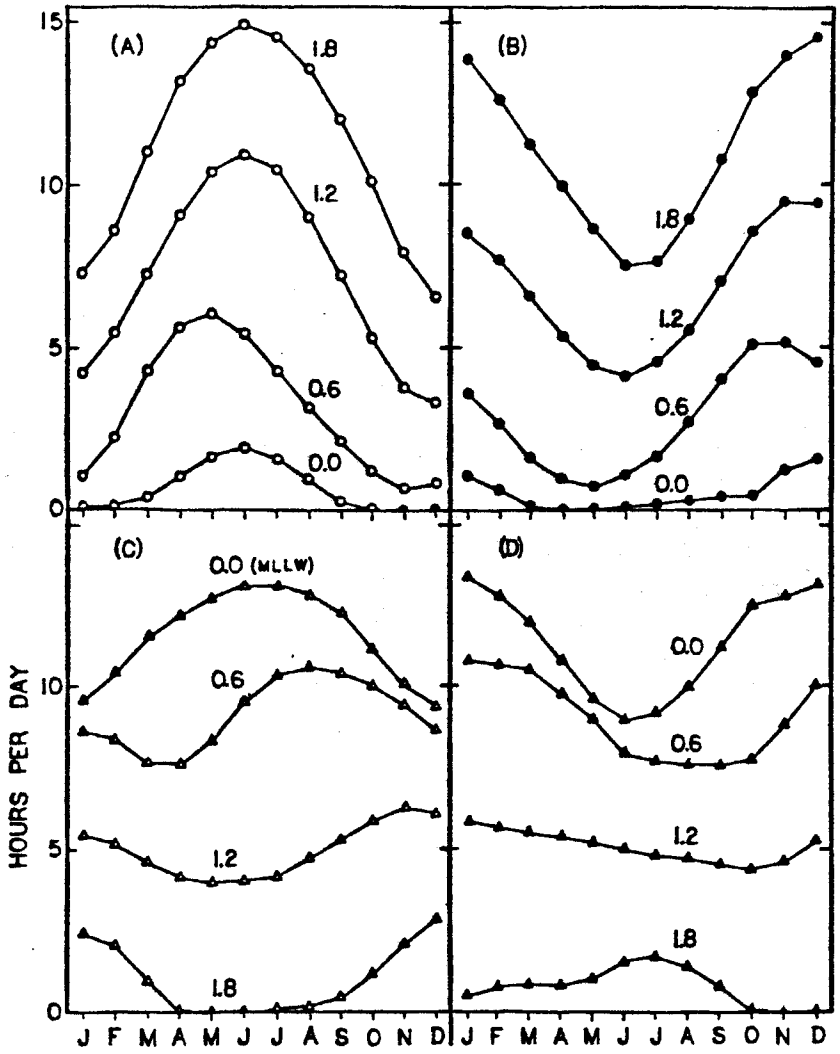
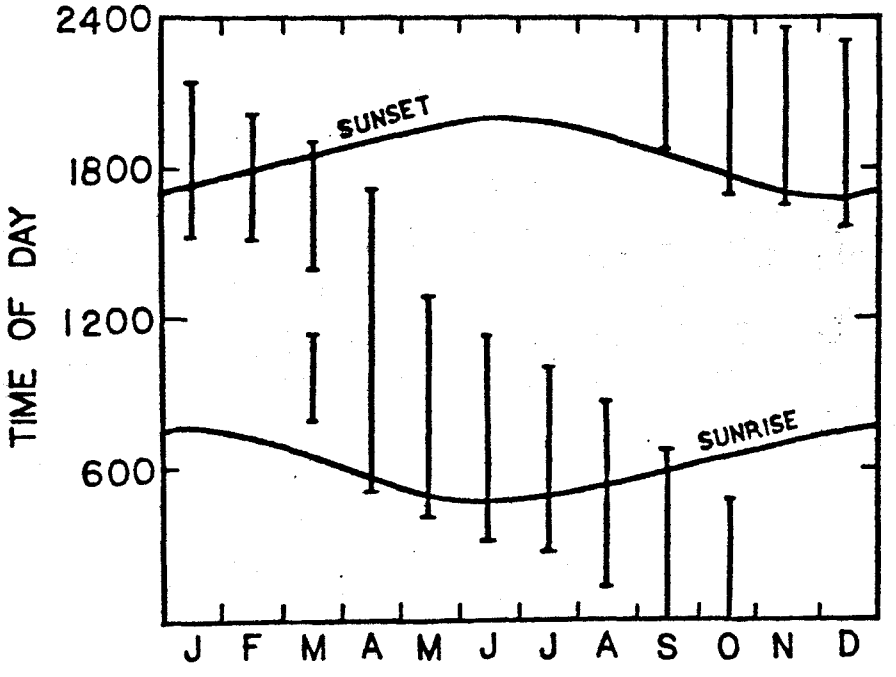

(A)
1.8
1.2
0.6
0.0
(B)
1.8
1.2
0.6
0.0
(C)
0.0 (MLLW)
0.6
1.2
1.8
(D)
0.0
0.6
1.2
1.8
HOURS PER DAY
15
10
5
0
J F M A M J J A S O N D
J F M A M J J A S O N D

Figure 32. Humboldt Bay: vertical bars for each month indicate the range of predicted times (Pacific Standard Time) for all low tides below MLLW, 1980-1983.


2400
1800
1200
600
TIME OF DAY
SUNSET
SUNRISE
J F M A M J J A S O N D

a slight advance in the tide pattern of about one hour per month during the summer and winter (Figure 32). However, during a single spring-neap-spring tide series at the vernal and autumnal equinoxes, the timing of the tide patterns advances about six hours. This sudden shift greatly increases the observed differences in exposure and submergence between winter and summer months. Regions without mixed tides may not experience this repeated annual pattern of extreme low or high waters (Bleakney, 1972).

It would seem upon first consideration that the time at which such very low tides occur will change more through the course of a month, for the tide cycle takes nearly 25 hours for completion. This time lag should bring the lower low tides into a different portion of the day. However, the cumulative lag is almost precisely matched by the successive alternation of the higher and lower low tides through a spring-neap-spring series (Figure 28B). The combined result maintains the time of day at which the extremely high or low tides occur during a given month (Figure 33).

At Port Townsend, the northernmost reference station considered, we observe very simple exposure and submergence patterns for daylight and darkness (Figure 34). The larger annual changes in daylength at this latitude are readily apparent. The tide levels for which information is presented are not strictly comparable to those same levels from the other reference stations, for the tidal amplitude increases with latitude. Nonetheless, the distinct patterns will hold for levels both above and below those presented. Note also that the phase-shifted

Figure 33. Humboldt Bay: tidal topographic map for May 1982, with a contour interval of 0.6 m and a time-interval accuracy of 1 hour. Stippled areas indicate tide levels below MLLW; plus (+) signs indicate tide levels above 1.8 m. Times of sunrise and sunset are indicated. Note the occurrence of two high tide "ridges" and two low tide "valleys" each day (read vertically), the lag time of roughly one hour per day in the tide pattern, and how the progression of the tides causes the extremely low tide levels to recur during daylight hours in this month.

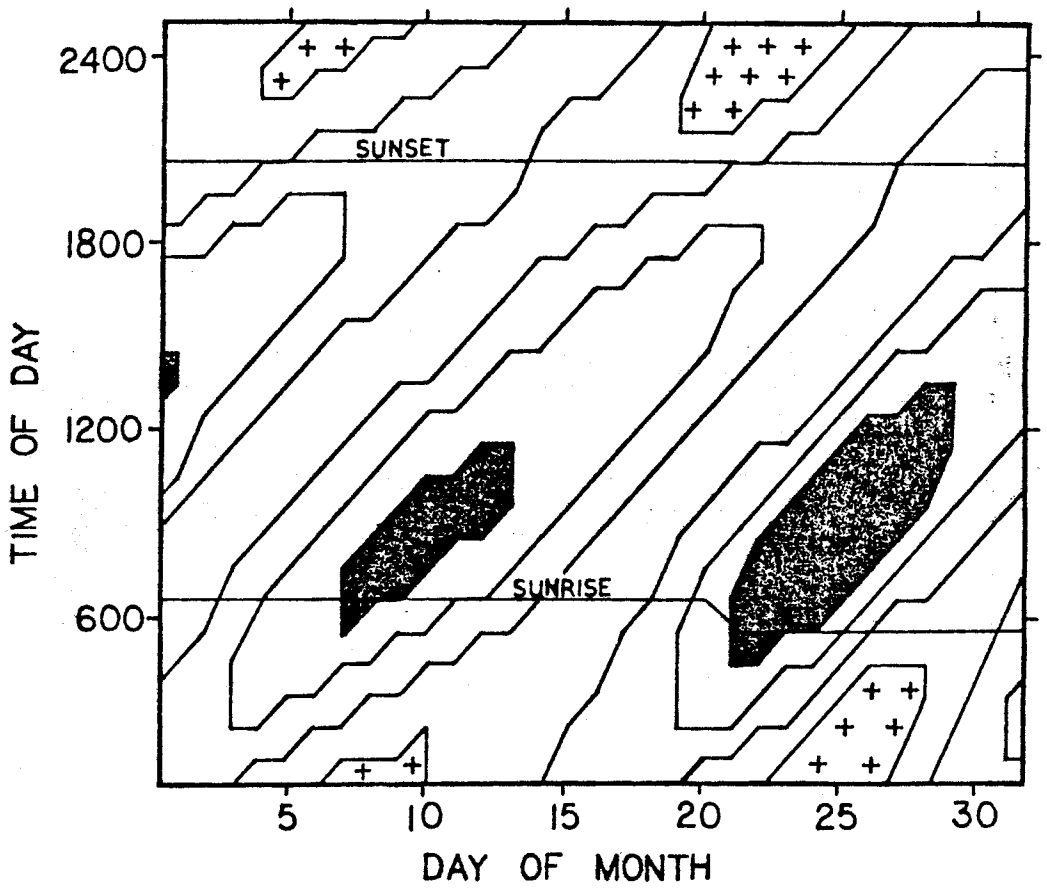
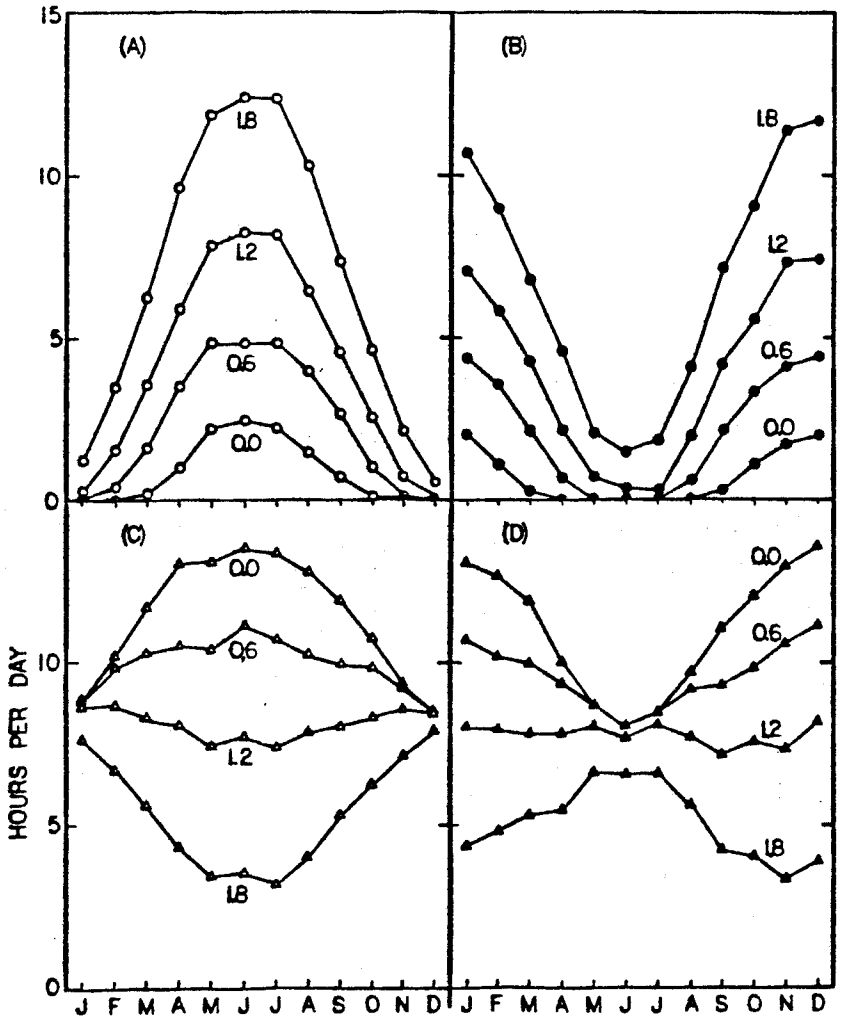

2400
1800
1200
600
TIME OF DAY
SUNSET
SUNRISE
5
10
15
20
25
30
DAY OF MONTH

Figure 34. Port Townsend: daily durations (month average) of emergence (circles) and submergence (triangles) in daylight (open circles) and darkness (filled circles) at 0.6 m intervals between MLLW and 1.8 m: (A) daylight exposure (B) night exposure (C) daylight submergence (D) night submergence.


(A)
(B)
(C)
(D)
1.8
1.2
0.6
0.0
HOURS PER DAY
15
10
5
0
J F M A M J J A S O N D

asymmetry of the intermediate levels is reduced. This is due to the temporal interaction between the tidal rhythms and the daylength patterns; the extremely low tides occur at midday around the summer solstice and at midnight around the winter solstice.

The peculiar timing of tidal and daylength cycles is well known and documented for particular locations. Sousa (1979) indicates that low tides below MLLW occur during daylight hours "between the months of October and March" for a site near Santa Barbara, California (34° 25' N); Sutherland (1970) finds that such daytime low tides occur from February to May at Bodega Head, California (38° 18' N); Dayton (1971, 1975) indicates that extremely low tides occur around midday during summer months in the San Juan Islands, Washington (48° 30' N), not far from the Port Townsend reference station. On any given day, the tide-wave form sweeps along the Pacific coast of North America, which produces a time lag of some hours between the occurrence of low tide at San Diego and low tide at Humboldt Bay, and a further lag before low tide at Port Townsend. This difference of a few hours per day results in the difference of a few months in the occurrence of extremely low tides at midday.

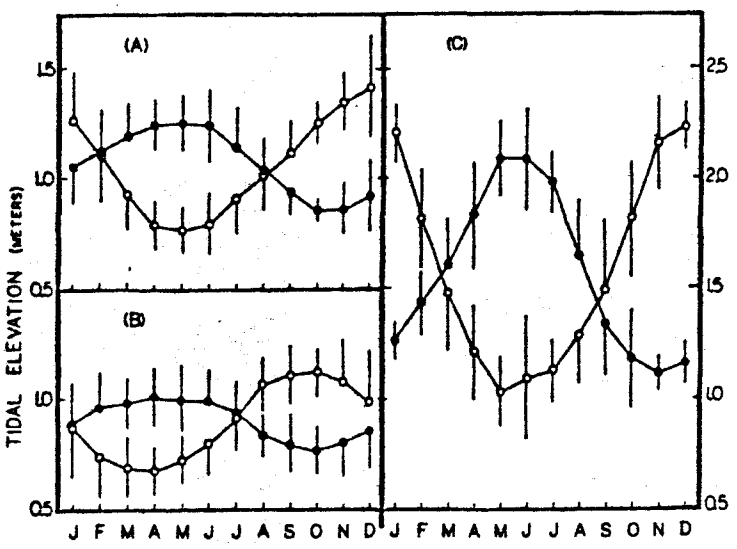
Similar lags of a few hours occur as the tide procedes up estuaries. This should cause a shift of a few months in the day-night submergence-emergence patterns over a small distance as compared with those between the reference stations considered here. While daylength changes will be unimportant over such short distances, the tidal lag of several hours may be sufficient to alter the timing of estuarine biological

processes. Maximum benthic algal production that is correlated with tidal phenomena (e.g., Shellum and Josselyn, 1982) may occur in different months at different locations within an estuary. Shorebirds may switch preferences for certain feeding areas, with possible effects on the benthic fauna from the changing predation pressures.

There is a natural human bias in the study of intertidal environments towards those processes or interactions that are more obvious in daylight exposure. Our subjective attention to the intertidal zone may change seasonally owing to the patterns detailed above; we are simply more likely to see most of the intertidal at certain times of the year (Figure 35). At Humboldt Bay, the central reference station considered here, daylight water levels are lowest in late spring and early summer and highest in fall and midwinter (Figure 35A). At San Diego, the pattern is similar (Figure 35B), but moderated by the smaller tidal range and shifted a month or so earlier (owing to the tidal lag phenomenon previously discussed). An anticipated, Port Townsend shows a more distinct annual pattern, which is caused by its greater tidal range (Figure 35C).

One should consider several cautions before applying such information in predictive quantitative studies. First, the computer programs that have b-en assembled produce estimates of exposure and submergence that are based upon (1) predicted rather than actual times and heights of the tides, and (2) a simple sine curve interpolation that may not mimic the true fluctuations of water levels in coastal or estuarine areas. Factors such as changing barometric pressure, shifts

Figure 35. Mean water levels in daylight (open circles) and darkness (filled circles): monthly mean ± 1 s.d. calculated from daily means for (A) Humboldt Bay (B) San Diego (C) Port Townsend.


(A)
(B)
(C)
TIDAL ELEVATION (METERS)
1.5
1.0
0.5
2.5
2.0
J F M A M J J A S O N D J F M A M J J A S O N D

in wind direction, and waves that strike shores of different slopes may create substantial differences between predicted and actual water levels (Druehl and Green, 1982). Secondly, the use of monthly mean values will indicate general patterns, but there is substantial day-to-day variation in emergence at the high and lower tidal elevations. Extreme elevations or maximal durations of exposure and submergence are more likely to affect biological activities than mean values (Bleakney, 1972; Wolcott, 1973; Swinbanks, 1982). Interestingly, the differences in exposure and submergence over short vertical intervals are greater during neap tides, when one might expect more moderating conditions, and smaller during spring tides, when one might expect more extreme changes. Spring tide conditions will have a greater effect upon the lower intertidal environment, while neap tide conditions increase exposure in the higher intertidal areas. The stratification of surface waters in estuaries is also stronger during neap tide series, with greater differences in temperature and salinity over short vertical distances in the water column (Haas, 1977); the greater tidal flux during spring tide series increases turbulent mixing and creates a more homogeneous water column. However, this shortcoming of the data presented here can be overcome by slight modification of the computer programs; this will permit calculation of the maximum or minimum values of day or night emergence and submergence. Wave splash in exposed intertidal locations will blur the distinction between emergence and submergence and will moderate potential stresses of desiccation and rainfall. Different hours of daylight exposure are not necessarily equivalent for many organisms owing to variable insolation caused by patchy

cloud cover and the daily and seasonally changing position of the sun in the sky. Wave-induced turbidity in shallow water may affect light-dependent processes during daylight submergence.

Despite these limitations, the results provided by these analyses should prove useful for several types of studies. Investigations of the physiological or behavioral responses of organisms that occur over a broad vertical range in the intertidal zone may find that the quantitative assessments provided by this approach will indicate limits for some activity of interest. Alternatively, if the organism under scrutiny is found to change vertical position seasonally or clinally over its range (along a coastline or up an estuary), these programs may provide insights through their ability to include correction factors of tidal time and height away from central reference stations. Organisms that occur over large latitudinal ranges may show changes in the timing of reproduction or of settlement of planktonic propagules; the predictable shifts in the annual pattern of diurnal emersion suggest yet another underlying selection parameter. Mobile organisms that can readily change their use of intertidal habitats, such as bottom-feeding fishes and migratory waterfowl, may show distributional changes over short and long time scales predicted by results from these programs. Finally, this information is of intrinsic value to the student of intertidal biology, who merely wishes to know how long study sites at particular elevations will remain accessible for examination.

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